

EVALUATION OF PUTRESCINE, CADAVERINE, AND INDOLE AS
CHEMICAL INDICATORS OF DECOMPOSITION IN PENAEID SHRIMP

By

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The US Food and Drug Administration (FDA) has traditionally used a three-class system based on sensory analysis to evaluate decomposition in shrimp. Additionally, decomposition in shrimp has been chemically confirmed by the presence of indole concentrations of 25 μ g/100g or greater. However, the FDA revoked Compliance Policy Guide 7108.11, which supported indole at 25 μ g/100g as confirmatory for decomposed shrimp, because it was subject to misinterpretation and did not reflect agency policy. Further research was necessary to resolve questions concerning the utility of indole and alternative chemical indicators for shrimp decomposition. Possible alternatives include putrescine and cadaverine, which have been shown to increase as spoilage progresses.

The proposed hypothesis was that putrescine and/or cadaverine may be better chemical indicators of decomposition than indole for decomposed shrimp. The

experimental approach was to evaluate putrescine, cadaverine, and indole as chemical indicators of decomposition, to determine the levels at which these indicators confirm sensory decomposition, and to identify microorganisms associated with the production of these metabolites in decomposing Penaeid shrimp.

Wild-caught domestic white shrimp (*Litopenaeus setiferus*), pink shrimp (*L. brasiliensis*), and Nicaraguan aquacultured white shrimp (*L. vannamei*) were decomposed at 0, 12, 24, and 36°C. Aerobic plate counts with microbial type characterizations were determined for shrimp at selected decomposition times. Sensory, putrescine, cadaverine, and indole analyses were conducted for each decomposition time. Additionally, commercial shrimp samples collected for sensory evaluation by the FDA were tested for putrescine, cadaverine, and indole.

Decomposition progressed more rapidly at higher temperatures. Bacteria capable of producing putrescine, cadaverine, and indole increased as decomposition temperatures increased and putrescine, cadaverine, and indole levels generally increased with time and temperature.

Overall, this study demonstrated that putrescine concentration at the 3-ppm level was the most effective chemical indicator for decomposition in shrimp decomposed at 0, 12, 24, and 36°C and in field verification studies with FDA samples. It consistently confirmed sensory decomposition more accurately than cadaverine concentration at 3 ppm, indole concentration at 3 µg/100g, and indole concentration at 25 µg/100g. Thus, it is recommended that putrescine concentration at the 3-ppm level be incorporated in industry and regulatory analyses to confirm (pass vs. fail) sensory decomposition in Penaeid shrimp.

INTRODUCTION

Decomposition, as defined by the Food and Drug Administration (FDA), “consists of the bacterial breakdown of the normal product tissues and the subsequent enzyme-induced chemical changes. These changes are manifested by abnormal odors, taste, texture, color, etc.” (FDA, 1998, p. 3). Regulations associated with decomposition are found in Sec. 402 of the Federal Food, Drug, and Cosmetic Act (FD&C Act) which focuses on adulterated food. In shrimp decomposition, Sec. 402 (a) (3), which states that a food is adulterated “if it consists in whole or in part of any filthy, putrid, or decomposed substance, or if it is otherwise unfit for food,” and Sec. 402 (a) (4), which states that a food is adulterated “if it has been prepared, packed, or held under unsanitary conditions whereby it may have become contaminated with filth, or where by it may have been rendered injurious to health,” are of primary importance (Food and Drug Law Institute, 1998). The FDA uses sensory analyses to determine if shrimp is adulterated under the FD&C Act as a result of decomposition.

Sensory evaluation involves the employment of one or more of the physical senses (sight, touch, taste, smell) for subjective testing and rating of food products. Seafood sensory analyses as conducted by the FDA are primarily concerned with the smell or odor characteristics of the product. Traditionally the FDA has employed a three-class system for the evaluation of domestic and imported shrimp and other seafood products. Within this system Class 1 (Passable) shrimp possess very fresh

odors or other odors characteristic of the product that are not identifiable as decomposition. Class 2 (Decomposed-Slight but definite) includes shrimp with a slight but definite odor of decomposition. The odor that is detected, although not necessarily intense, is persistent and readily identifiable to the experienced analyst as that of decomposition. Class 3 (Decomposed-Advanced) shrimp possess the distinct and unmistakable odors of decomposition (Code of Federal Regulations, 1991). This evaluation is based solely on subjective sensory (odor) characteristics of the shrimp as determined by trained sensory analysts.

Until 1996 Class 2 decomposition in shrimp was confirmed by indole concentrations in shrimp greater than or equal to $25\mu\text{g}/100\text{g}$ but less than $50\mu\text{g}/100\text{g}$ and Class 3 decomposition was confirmed by indole concentrations greater than or equal to $50\mu\text{g}/100\text{g}$ (FDA, 1996b). However, Compliance Policy Guide 7108.11 which supported indole concentration at the $25\text{-}\mu\text{g}/100\text{g}$ level as confirmatory for decomposed shrimp was revoked due to confusion about its application by industry and the FDA (FDA, 1996a). This confusion stemmed from the fact that the absence of indole does not mean that shrimp is acceptable. Shrimp may be judged to be decomposed and thus considered adulterated based on sensory evaluation without the presence of indole at $25\mu\text{g}/100\text{g}$ (FDA, 1995). Indole is a limited chemical indicator of decomposition in shrimp, especially when the shrimp are decomposed at low temperatures. Research has shown that it is possible to have clearly decomposed shrimp with indole concentrations less than $25\mu\text{g}/100\text{g}$ (Chang et al., 1983; Shamshad et al., 1990).

The issues stated above present the need to resolve questions concerning the utility of indole analysis and alternative chemical indicators for shrimp decomposition. Possible alternatives include putrescine and cadaverine, which have been shown to increase as decomposition in shrimp progresses (Shakila et al., 1995). Our response was to formulate a scientific investigation into these issues with the following objectives: (1) to determine the odor, indole, amine, and microbiological characteristics of Penaeid shrimp decomposed at temperatures ranging from 0-36°C, (2) to evaluate the efficacy of putrescine, cadaverine, and indole as chemical indicators of decomposition for Penaeid shrimp, (3) to determine the concentration(s) at which each chemical indicator confirms sensory decomposition, (4) to assess the feasibility of using putrescine/cadaverine analysis to supplement or improve the current decomposition evaluation system, (5) to determine the predominant microflora present and the microorganisms responsible for putrescine, cadaverine, and indole production throughout temperature dependent decomposition, and (6) to verify the efficacy of putrescine, cadaverine, and indole as shrimp decomposition indicators using commercial samples collected and organoleptically evaluated by FDA experts.

REVIEW OF LITERATURE

Measures for Decomposition

The United States Food and Drug Administration has established a three-class rating protocol for the evaluation of decomposition in domestic and imported shrimp. Class 1 (Passable) shrimp possess very fresh odors or other odors characteristic of the product that are not identifiable as decomposition. Class 2 (Decomposed-Slight but definite) includes shrimp with a slight but definite odor of decomposition. The odor that is detected, although not necessarily intense, is persistent and readily identifiable to the experienced analyst as that of decomposition. Class 3 (Decomposed-Advanced) shrimp possess the distinct and unmistakable odors of decomposition (Code of Federal Regulations, 1991). This evaluation is based solely on sensory (odor) characteristics of the shrimp as determined by trained personnel.

Early research focused on the need to supplement the subjective organoleptic analyses with more objective and precise quality analyses based on chemical and/or microbiological characteristics of the shrimp (Fieger and Friloux, 1954; Bailey et al., 1956). As a result, numerous tests have been proposed as shrimp quality indicators. Some of these tests included pH, glycogen-sugar, lactic acid, trimethylamine (TMA) (Bailey et al., 1956), volatile reducing substances (VRS) (Farber, 1954), amino acid nitrogen (AA-N), total volatile nitrogen (TVN), TVN/AA-N ratio (Cobb et al., 1973a; Cobb and Vanderzant, 1975), adenosine triphosphate degradation products (Flick and

Lovell, 1972), adenosine monophosphate deaminase activity (Finne, 1982), and indole (Chang et al., 1983). Unfortunately, none of these methods are generally accepted as a sufficient decomposition measure or quality index for shrimp.

In the past, the Food and Drug Administration has used quantitative indole analysis to confirm organoleptic evaluations of decomposed shrimp. Indole levels of less than 25 μ g/100g confirmed Class 1 decomposition, greater than or equal to 25 μ g/100g but less than 50 μ g/100g denoted Class 2 decomposition, and greater than or equal to 50 μ g/100g confirmed Class 3 decomposition (FDA, 1996b). This information was taken from Compliance Policy Guide (CPG) 7108.11 that was recently revoked by the FDA (FDA, 1996a).

The FDA has recently revoked two CPG's involving decomposed shrimp and indole analyses. First, on July 5, 1995, CPG 7119.13, "Canned and Cooked/Frozen Shrimp - Adulterated by Decomposition," was revoked in order minimize confusion resulting from industry misinterpretation (FDA, 1995). Apparently, the industry took the position that shrimp with indole levels less than 25 μ g/100g were considered acceptable regardless of organoleptic analysis results, which was contrary to FDA policy. CPG 7119.13 offered guidance to FDA personnel in evaluating canned and cooked/frozen shrimp imported into the United States. The revocation stated that "The mere absence of indole does not mean, however, that the shrimp is acceptable. Organoleptic analysis can also be used to determine whether the shrimp is adulterated." It also states that the "FDA intends to use any appropriate methods of analysis for examining canned and cooked/frozen shrimp offered for import" (FDA, 1995).

Secondly, on December 24, 1996, CPG 7108.11, "Shrimp – Fresh or Frozen, Raw, Headless, Peeled or Breaded – Adulteration Involving Decomposition," was revoked as a result of similar confusion in interpreting organoleptic and indole analyses of shrimp by individuals outside and within the FDA (FDA, 1996a). This was the primary CPG for assessing decomposition in shrimp. It was also the source of the above-mentioned levels for indole confirmation of Class 1, 2, and 3 shrimp. In this revocation the FDA states, "Until such time as the agency develops appropriate new guidance, it intends to use any appropriate method of analysis for examining shrimp and to review recommendations for regulatory action against decomposed shrimp on a case-by-case basis" (FDA, 1996a). Therefore, a need to supplement the subjective organoleptic analyses with more objective and precise quality analyses based on chemical and/or microbiological characteristics of shrimp exists.

Components of Decomposition

Decomposition of seafood products is primarily the result of bacterial degradation of soluble low molecular weight nitrogenous compounds that are commonly referred to as non-protein nitrogen. It is generally accepted that microbiological spoilage or decomposition in meat and seafood products takes place primarily in the juices containing the soluble low molecular weight free amino acids, peptides, and other non-protein nitrogen components. These compounds are broken down into typical spoilage metabolites including indole, ammonia, putrescine, and cadaverine (Eskin et al., 1971). This occurs, especially at low temperatures, without any significant degradation in the structure of primary proteins – at least not complete degradation (Jay, 1996).

Support for this theory has been demonstrated via total protein analyses on spoiled and fresh beef (Jay and Kontou, 1967). Additionally, analyses of the extract-release volume (ERV) of beef revealed that as microbial spoilage progressed, the ERV did not increase as would be expected with complete protein hydrolysis, but instead decreased (Jay, 1964). ERV is the measure of the volume of liquid released from a tissue homogenate after filtering for a given time period (Jay, 1996).

Lerke et al. (1967) separated clarified fish press juice into protein and non-protein (protein-free) fractions by gel filtration. Free amino acids and peptides characterized the non-protein nitrogen fraction. After inoculation with spoilage organisms and incubation at 22°C for 24 hr, it was shown that raw fish juice, cooked fish juice, and the non-protein nitrogen fraction displayed spoiled odors and high levels of volatile reducing substances (VRS) and trimethylamine (TMN). However, no spoilage was detected in the protein fraction. Significant proteolysis was not apparent until after spoilage was evident. The protein fraction does, however, support bacterial growth with little evidence of spoilage. The advanced stages of decomposition (putrefaction), after the free amino acids, peptides, and nucleotides have been nearly exhausted, are characterized by proteolytic deterioration of proteins (Eskin et al., 1971). Non-protein nitrogen compounds are significant in fish and shellfish because they contribute to flavor and serve as primary substrates for microbial spoilage organisms (Eskin et al., 1971; Finne, 1992). The non-protein nitrogen fraction of shrimp consists of free amino acids (65%), peptides (15%), nucleotides (5%), trimethylamine oxide (TMAO) (5%), and betaines (10%) (Finne, 1992).

Free Amino Acids

Free amino acids that occur at high levels in crustaceans include alanine, arginine, taurine, proline and glycine. Crabs exhibit higher levels of taurine, whereas shrimp have high levels of glycine (Konosu and Yamaguchi, 1982). Free amino acids like glycine and alanine contribute sweetness to the flavor of shrimp, while proline contributes a desirable characteristic. Alanine contributes less to sweetness than does glycine (Konosu and Yamaguchi, 1982). Glycine, alanine, proline, arginine, and serine/threonine make up greater than 90% of the free amino acid pool in shrimp (Papadopoulos and Finne, 1986).

Research investigating the effects of environmental salinity on sensory characteristics of penaeid shrimp showed that shrimp adapted to higher salinities displayed higher concentrations of amino acids in their tissues as a result of osmoregulation (Papadopoulos and Finne, 1985, 1986). Flavor evaluations showed that shrimp grown at different environmental growth salinities imparted significant variations in flavor characteristics. Shrimp grown at higher salinities were sweeter, shrimpier, nuttier and saltier than those grown at lower salinities. It was determined that more flavorful shrimp could be produced with growth at high environmental salinities (Papadopoulos and Finne, 1986).

Indole

Bacterial deamination of the free amino acid tryptophan results in the production of indole, pyruvate, and ammonia (Eskin et al., 1971). Indole is accepted as a good indicator of high temperature decomposition in shrimp (Nickelson, 1992). Duggan and Strasburger (1946) stated that research conducted by the FDA in the

mid-1930's indicated that indole could be used as a decomposition index in shrimp. Then after extensive studies, these authors in turn concluded that indole levels in shrimp were an indication of the degree of decomposition but that decomposed shrimp may not contain appreciable amounts of indole (Duggan and Strasburger, 1946). Salwin (1964) reported that, when compared to seven other chemical tests, acceptable and unacceptable shrimp were best differentiated by indole concentration. More recent research supports the conclusions of Duggan and Strasburger (1946) showing that low decomposition temperatures result in clearly decomposed shrimp with indole levels below 25µg/100g, the concentration that confirms decomposition (Chang et al., 1983; Shamshad et al., 1990). These findings suggest an apparent inadequacy in the use of indole as an overall decomposition indicator (Chang et al., 1983; Shamshad et al., 1990).

High indole levels in shrimp decomposed at elevated temperatures result from the growth of mesophilic microorganisms, which frequently possess the ability to convert tryptophan to indole (Chang et al., 1983; Matches, 1982; Smith et al., 1984; Shamshad et al., 1990). While investigating the effects of temperature on decomposition of the Pacific shrimp (*Pandalus jordani*), Matches (1982) found that at higher decomposition temperatures (11.1, 16.7, and 22.2°C) accelerated indole production was associated with the emergence of *Proteus*. The above study showed that indole levels increased with time in shrimp incubated at all temperatures (including 0 and 5.6°C), even though *Proteus* species were not detected.

Smith et al. (1984) isolated 1,647 bacterial colonies from white shrimp (*Penaeus setiferus*) homogenate and found that 2.6% of these organisms were indole

producers. The majority of isolates that produced indole in shrimp homogenate were proteolytic *Aeromonas* and *Proteus* that had minimum temperatures for indole production of 22°C. Proteolytic activity was considered necessary to liberate free tryptophan from tissue proteins. These organisms were associated with high temperature indole production. Several of the indole positive isolates (52.4%) were found to be psychrotrophic, non-proteolytic *Flavobacterium* that could only produce indole in shrimp homogenates with added tryptophan. These organisms were associated with low temperature indole production. This phenomenon would probably only occur in the later stages of spoilage when proteolytic *Pseudomonas* became dominant and produced free tryptophan. Additionally, indole production in shrimp was found to be related to bacterial activity as opposed to endogenous enzymatic activity.

Chang et al. (1983) investigated the effect of incubation temperature on the formation of indole in pink (*Penaeus duorarum*) and white (*Penaeus setiferus*) shrimp, as well as the effects of freezing and boiling on indole stability. Rapid indole formation was evident at 12 and 22°C. At 4°C indole formation reached a maximum of 18 µg/100g at day 13 and showed a value of less than 10 µg/100g on day 8 when the shrimp was considered spoiled by APC (10^8 /g) and TVN (56 mg TVN-N/100g) parameters. Frozen storage of shrimp with known indole levels at -26°C showed indole to be stable over a 6-month storage period. Additionally, boiling of decomposed shrimp for 5 minutes caused a slight decrease in indole levels but not to the degree as to alter the original classification of the shrimp. Boiling of acceptable

shrimp had little to no effect on indole concentration. The above study demonstrated the usefulness of indole as an indicator of high temperature decomposed shrimp.

In a study on the shelf life of Pakistan shrimp (*Penaeus merguensis*) at temperatures ranging from 0-35°C, Shamshad et al. (1990) found that indole production was well correlated ($r = 0.88$) with the emergence of *Vibrio* spp. which became the dominant microflora only at temperatures of 10°C and above. Indole levels at 0 and 5°C reached only 4.9 µg/100g by day 16 and 4.2 µg/100g by day 12, respectively, which again is well below 25 µg/100g. The predicted shelf life of these shrimp stored at 0 and 5°C was 13 and 9 days, respectively. The authors stated that very low indole levels could be detected during the sensory shelf life of the shrimp at all temperatures. The 35°C storage temperature was the only temperature at which the indole level exceeded the FDA suggested limit of 25 µg/100g before the shrimp were considered spoiled by sensory analysis, APC, TVB, and TMN-A. This research again shows indole analysis of shrimp to be a good indicator of decomposition but that decomposed shrimp do not necessarily have to contain elevated indole levels.

The above discussion clearly indicates that the indole level in shrimp is of limited value as a chemical indicator of decomposition. This in turn suggests that a chemical indicator for shrimp decomposition that is functional and effective over the wide range of possible spoilage temperatures would be of more value to the shrimp industry and regulatory authorities.

Amines

Amines are typically produced in shrimp by the bacterial deamination or decarboxylation of free amino acids present in shrimp tissue. Deamination of amino

acids results in the formation of ammonia (NH_3) and organic acids, while decarboxylation leads to the production of amines and carbon dioxide (Eskin et al., 1971; Finne, 1992). Breakdown of trimethylamine oxide (TMAO) may also result in the formation of trimethylamine (TMA) and dimethylamine (DMA) in seafood products (Regenstein et al., 1982). Ammonia, TMA, and DMA are volatile amines with odor thresholds shown in Table 1. Biogenic amines like putrescine, cadaverine, histamine, spermidine, spermine, tyramine, and tryptamine are nonvolatile amines that may also be produced during decomposition of seafood products (Rawles et al., 1996).

Table 1 - Odor thresholds of volatile amines

Volatile Amine	Parts Per Billion
Ammonia	110,000
DMA	30,000
TMA	600

Source: Regenstein et al., 1982

Volatile Amines

Bacterial deamination may progress in numerous ways depending on the microbial types present on the shrimp and the time and temperature of storage (Finne, 1992). Ammonia is a product of this deamination regardless of the mechanism of reaction and as such has been suggested as an indicator of shrimp quality (Luzuriaga et al., 1997). Shrimp were considered spoiled at the NH_3 level of 230 ppm (Cheuk and Finne, 1984; Luzuriaga et al., 1997). Additionally, ammonia was determined to be the primary component of the total volatile nitrogen (TVN) content of shrimp

(Cobb et al., 1973b). Montgomery et al. (1970) suggested a TVN value of 30 mg N/100g as indicative of shrimp spoilage.

Ammonia is also produced as a result of endogenous tissue enzymes present in shrimp (Finne 1982). Tissue enzymes associated with the production of ammonia in white shrimp (*Penaeus setiferus*) were determined to be adenosine deaminase and adenosine monophosphate (AMP) deaminase. Arginase was also shown to be present. It was postulated that arginase in combination with bacterial urease could be another source of ammonia in shrimp. Arginase breaks down arginine into ornithine and urea while urease converts urea to ammonia and carbon dioxide (Mathews and van Holde, 1990; Brock et al., 1994). Enzymatic ammonia production at temperatures ranging from 3-44°C for one day accounted for more than half of the total ammonia production in white shrimp demonstrating the importance of these enzymes relative to shrimp spoilage (Yeh et al., 1978). AMP deaminase activity has been suggested as an indicator of post-harvest storage time of iced shrimp (Cheuk et al., 1979).

In addition to ammonia; TMA, DMA, and other nitrogenous compounds may be components included as part of TVN analysis in seafood products. Total volatile base analysis includes primarily NH_3 , TMA, and DMA (Jay, 1996). TMA is a volatile amine with flavor and odor characteristics resembling that of ammonia (Finne, 1992). TMA primarily results from trimethylamine oxide reduction by enzymes of bacterial origin and may be produced in fishery products stored on ice and at refrigerated temperatures (National Research Council, 1985). Montgomery et al. (1970) have proposed an acceptability limit of 5 mg TMA-N/100g for shrimp in

Australian markets. In a study on shrimp (*Penaeus merguensis*) from Pakistan, it was stated that values of TMA-N ≤ 5 mg/100g combined with pH ≤ 7.5 , TVB ≤ 28.5 mg/100g, and indole ≤ 9.0 $\mu\text{g}/100\text{g}$ would probably represent acceptable quality shrimp (Shamshad et al., 1990). However, Cobb et al. (1973b) found that TMA could not be detected in freshly caught or spoiled brown shrimp (*Penaeus aztecus*) stored on sterile ice. Additionally, Chang et al. (1983) reported that TMA was not a good indicator of shrimp quality. In this study, TMA could not be detected in shrimp stored on ice prior to day 8 when the APC approached $10^8/\text{g}$ and the shrimp were of poor quality. By day 13 of iced-storage TMA-N/100g had only reached 3 mg which is below the 5 mg TMA-N/100g limit suggested for spoiled shrimp by Montgomery et al. (1970).

Another reaction involving the overall quality of seafood products is the enzymatic breakdown of TMAO. During frozen storage of gadoid fish, with microbiological activity minimized, TMAO may be degraded by endogenous enzymes to form equimolar portions of DMA and formaldehyde (FA) (Regenstein et al., 1982). Formaldehyde reacts with free amino groups of myofibrillar proteins resulting in protein deterioration and a subsequent degradation in textural quality (Finne, 1992). DMA can be used as a reliable quality index of gadoid fish in frozen storage (Hebard et al., 1982).

Biogenic Amines

Decarboxylation of free amino acids present in the tissue of fish and shellfish by bacterial enzymes leads to the formation of biogenic amines. There are large quantities of published research that address biogenic amine formation in food

products which include seafood, meats, cheeses, beers, wines, soybean products, and fermented vegetables (Shalaby, 1996). The bulk of this research in seafood products has been concentrated in the area of fish and fish products that have been associated with Scombroid or histamine poisoning. Fish in the families *Scombridae* and *Scomberesocidae* such as tuna, mackerel, and kingfish and non-scombroid fish like mahi mahi and marlin with high levels of free histidine have been implicated in cases of scombroid poisoning (Taylor et al., 1984). The presence of histamine is considered indicative of decomposition in tuna and mahi mahi (Rogers and Staruszkiewicz, 1997). As such, the FDA has established a defect action level of 50 ppm for tuna and mahi mahi (FDA, 1996c).

Putrescine and cadaverine are biogenic amines that have been identified as major products of decomposition in marine food products as well as meat and poultry (Jay, 1996). These diamines may play a role as potentiators of histamine toxicity by competing with histamine as substrates for diamine oxidase, the enzyme responsible for histamine metabolism (Taylor et al., 1984; Rawles et al., 1996). Putrescine is the decarboxylation product of ornithine while cadaverine is the decarboxylation product of lysine (Eskin et al., 1971). These diamines are considered useful indicators of decomposition because significant amounts of each are formed in numerous seafood products. The FDA has conducted a multi-laboratory collaborative study that was intended as a step toward setting defect action levels for putrescine and cadaverine for tuna and mahi mahi (Rogers and Staruszkiewicz, 1997).

Unlike the massive research base associated with biogenic amines in fish, very little research has been conducted with regard to their development in shrimp. Mietz

and Karmas (1978) analyzed the levels of putrescine, cadaverine, spermidine, spermine, and histamine in decomposed rockfish, salmon, lobster, and shrimp. An amine/polyamine index was developed to assess the degree of decomposition in these products relative to the organoleptic designations of Class 1, 2, and 3. The index value was determined using the formula listed below.

$$\frac{\text{Putrescine (ppm)} + \text{Cadaverine (ppm)} + \text{Histamine (ppm)}}{1 + \text{Spermidine (ppm)} + \text{Spermine (ppm)}}$$

This formula was developed because the authors noticed a general increase in putrescine, cadaverine, and histamine with decreases in spermidine and spermine as decomposition progressed. In composite test pack samples of four shrimp species, index values of 4.10, 8.93, and 67.80 were obtained for passable (Class 1), initial decomposed (Class 2), and advanced decomposed (Class 3) shrimp, respectively (Table 2). Index values of 0-5 were established for Class 1 shrimp while values of 5-25 represented Class 2 shrimp. Shrimp were considered to be Class 3 decomposed if the index value equaled or exceeded 25.

Table 2 - Analytical results (ppm) and classification of composite shrimp samples

Sam. ID	Put.	Cad.	His.	Spermi.	Sperm.	Index	Index Class	Organ. Rating	Total (put+cad+his)
SH1	3.44	2.00	0.22	0.22	0.16	4.10	1	1	5.66
SH2	27.90	7.80	0.38	0.58	2.46	8.93	2	2	36.08
SH3	60.38	7.42	0	0	0	67.80	3	3	67.80

Source: Mietz and Karmas, 1978

Analysis of individual amine data shows that putrescine was the dominant metabolite detected in the shrimp composites. It was also the only amine to clearly

distinguish between Class 1, 2, and 3 shrimp (Table 2). Each of the other amines displayed some variation in concentration relative to decomposition level. Cadaverine showed the ability to distinguish between Passable (Class 1) samples and Failed (Class 2 and 3) samples but could not separate Class 2 and 3 shrimp. These were however composite samples with undetermined product history. This suggests that putrescine and cadaverine may be good chemical indicators for decomposition in shrimp.

A second study investigated the development of putrescine, cadaverine, tyramine, APC, and sensory characteristics in Indian shrimp (*Parapenaeopsis stylifera*) that were decomposed with and without heads at 0, 5, and 30°C (Shakila et al., 1995). Putrescine levels were found to increase more rapidly than cadaverine or tyramine in whole or headed shrimp as decomposition progressed at all three temperatures tested. Increases in putrescine levels also correlated with decreases in sensory evaluations. It was concluded that putrescine was as a good indicator of spoilage in shrimp. This study demonstrates the potential for putrescine to be a reliable indicator for decomposition in shrimp regardless of the decomposition conditions encountered.

The occurrence of biogenic amines in scallop adductor muscle, raw surimi, flaked artificial crab, squid, shortneck clams, and boiled octopus has been investigated (Yamanaka, 1989; Hollingworth et al., 1990; Yamanaka et al., 1987; Nagayama et al., 1985). Yamanaka (1989) concluded that putrescine and ornithine have potential as effective indicators of squid freshness and that putrescine was the most useful indicator for squid freshness. Hollingworth et al. (1990) investigated

several potential chemical indicators of decomposition in raw surimi and flaked artificial crab at 4, 10, and 22°C. It was concluded that putrescine and histamine were unreliable indices because of relative insensitivity to progressive decomposition. This was presumed to be the result of limited quantities of ornithine. Cadaverine showed promise as a chemical indicator for this product however, concentrations did not begin to increase until after decomposition was apparent as determined by sensory analysis.

Agmatine, the biogenic amine produced by bacterial decarboxylation of arginine, has been suggested as a potential indicator of squid freshness (Yamanaka et al., 1987). In this study of squid decomposition at 0, 3.5, and 15°C, putrescine was not detected when squid was judged acceptable (Class 1) and appeared in low quantities (<10 ppm) when the squid reached the initial decomposition stage (Class 2). It was subsequently detected in large concentration in the advanced decomposition stage (Class 3). This indicates that putrescine would be a good indicator for decomposition in squid that was decomposed in the temperature range of 0 - 15°C. The authors stated that putrescine was a useful indicator for the initial decomposition level of squid. Nagayama et al. (1985) reported increases in the concentrations of putrescine, cadaverine, and tyramine in octopus and short neck clams decomposed at 27°C. Interestingly, a decrease in putrescine level was reported for the octopus decomposition from day 2 to day 3, when severe decomposition was apparent. An advanced decomposition decrease in putrescine was also reported by Shakila et al. (1995) for Indian shrimp (*Parapenaeopsis stylifera*) decomposed at 0 and 30°C.

Microbiology of Decomposition

The numbers and types of microorganisms present on shrimp determine the bacteriological quality of the product. The microflora present on fresh shrimp is generally a function of the microorganisms present in the water from which they are harvested, and handling procedures during harvest. Fresh shrimp from the Gulf of Mexico can be expected to have bacterial counts of 10^3 to 10^5 /g (Campbell and Williams, 1952; Vanderzant et al., 1970; Cobb et al., 1973b; Benner et al., 1994). Coryneforms, *Pseudomonas*, *Moraxella*, and *Micrococcus* species are usually predominant microflora in Gulf shrimp. Coryneforms generally dominate fresh shrimp while *Pseudomonas* species commonly dominate ice-stored shrimp (Vanderzant et al., 1970). Fresh Pacific shrimp (*Pandalus jordani*) are expected to have mean aerobic plate counts of approximately 10^6 /g (Harrison and Lee, 1968; Lee and Pfeifer, 1977; Matches, 1982). In order of predominance, the microbial flora of these shrimp consists primarily of *Moraxella*, *Acinetobacter*, *Flavobacterium*, and *Pseudomonas* (Harrison and Lee, 1968).

Vanderzant et al. (1970) investigated the microbial flora of Gulf of Mexico and Texas pond-raised shrimp. These authors found that bacterial counts were higher for Gulf shrimp ($\approx 10^4$ /g) than for white pond shrimp ($\approx 10^3$ /g). This difference was attributed to variation in environment and elimination of the extensive handling and storage on the boat. As stated above coryneforms, *Pseudomonas*, *Moraxella*, and *Micrococcus* species were the predominant microflora in Gulf shrimp while coryneforms, *Flavobacterium*, *Moraxella* dominated on sea water based media and coryneforms, *Bacillus*, and *Lactobacillus* were predominant on distilled water based

media. It was also noted that pond shrimp contained fewer *Pseudomonas* spp. (mean = 2.6%) than Gulf shrimp (mean = 18-22%).

Another investigation from the same ponds focused on the microflora of pond-raised brown shrimp (*Penaeus aztecus*) (Vanderzant et al., 1971). These shrimp had APC's ranging from $5 \times 10^4/\text{g}$ – $5.5 \times 10^6/\text{g}$ with coryneforms and *Vibrio* as the predominant microorganisms. Coryneforms, *Flavobacterium*, *Moraxella*, and *Bacillus* dominated the pond water microflora. Storage of these shrimp at 3-5°C for 14 days resulted in 50% acceptability as judged by organoleptic analysis. Of primary importance is the fact that *Pseudomonas* species were not significant contributors to the microflora of fresh or stored shrimp. Unfortunately, the microbial type distribution of these pond-raised shrimp was not presented. This would have given more insight into the details of microflora variation between wild and pond-raised brown shrimp stored at refrigerated temperatures.

Vanderzant et al. (1973) conducted another study which addressed microbial, chemical, and shelf life aspects of four species of pond-raised shrimp (*Penaeus vannamei*, *P. occidentalis*, *P. aztecus*, and *P. setiferus*). Fresh shrimp generally showed APC's of 10^4 to $10^5/\text{g}$ that was dominated by *Aeromonas*, *Pseudomonas*, and *Vibrio*. This differs from the previous study where coryneforms and to a lesser extent *Vibrio* dominated the initial microflora (Vanderzant et al., 1971). *Aeromonas* and *Pseudomonas* dominated the microflora on *P. vannamei* at days 7 and 14 of refrigerated storage. For all species of shrimp, coryneforms became predominant on day 21 followed by *Acinetobacter* and *Pseudomonas* on day 28. Total volatile nitrogen (TVN) levels for pond-raised shrimp and wild Gulf shrimp were comparable

while the free amino acid nitrogen was almost double for pond shrimp. This was attributed to the supply of protein-rich food in the ponds. It was important to note that the lysine-ornithine level increased from 0.36 mM/100g on day 0 to 1.14 mM/100g on day 14. This increase in lysine-ornithine concentration was attributed to ornithine production by arginase activity. The lysine-ornithine then decreased from 1.14 mM/100g on day 14 to 1.03 mM/100g by day 17 and 1.09 mM/100g by day 21. This could have been due to psychrotrophic microbial breakdown of lysine to cadaverine or ornithine to putrescine. The shrimp were considered spoiled by odor and appearance by day 17-21 of storage. Unfortunately, no microbial data was available for day 17 of storage.

Christopher et al. (1978) studied the microbiology of pond-raised shrimp (*Penaeus stylirostris*, *P. vannamei*, and *P. setiferus*) but this time the research was conducted in ponds from a different location. They found APC's for fresh shrimp ranging from 1.5×10^3 to 1.3×10^4 /g with coryneforms and *Vibrio* as dominant microflora. Pond water APC's ranged from 6.1×10^2 to 2.2×10^4 /ml with *Vibrio* as the dominant species. *Penaeus setiferus* and *P. vannamei* stored on ice for 8 days were both dominated by coryneforms and *Pseudomonas*.

Formation of metabolites like amines and indole in foods is regarded as a function of the microbial numbers and types as well as the time/temperature of storage. Controlled decomposition research on shrimp conducted by Matches, (1982) and Shamshad et al. (1990) showed that *Pseudomonas* species were dominant at low storage temperatures. More importantly, however, Shamshad et al. (1990) differentiated the pseudomonads into Group I, Group II, and *Alteromonas* (formerly

Pseudomonas group IV). This is important because *Alteromonas putrefaciens* is a psychrotrophic ornithine decarboxylase positive microorganism associated with low temperature seafood spoilage (van Spreekens, 1977; von Graevenitz, 1995). *Alteromonas putrefaciens* has now been renamed *Shewanella putrefaciens* (MacDonell and Colwell, 1985). Suzuki et al. (1988) determined that non-halophilic *Pseudomonas* III/IV, *P. fluorescens*, and *Alteromonas putrefaciens* were all significant putrescine producers but were very limited cadaverine producers. Okuzumi et al. (1990) in a study of horse mackerel stored at 5 and 30°C found that *Pseudomonas* III/IV-NH (non-halophilic) decarboxylated ornithine, *Pseudomonas* I/II-NH decarboxylated arginine, *Photobacterium* decarboxylated arginine and lysine, and *Vibrio* decarboxylated arginine. This suggests the possibility of a synergistic relationship between the ornithine producing Group I/II pseudomonads and the ornithine decarboxylating Group III/IV and *Alteromonas* which could result in a rather large psychrotrophic production of putrescine. *Pseudomonas* Group I/II, Group III/IV, and *Alteromonas* have all been isolated from low temperature stored shrimp (Lee and Pfeifer, 1997; van Spreekens, 1977; Shamshad et al., 1990). Perhaps these organisms are the source of putrescine in the Indian shrimp (*Parapenaeopsis stylifera*) that were decomposed at 0 and 5°C (Shakila et al., 1995).

Shakila et al. (1995) also demonstrated rapid putrescine production in shrimp stored at 30°C. Microbiological type distributions of Pakistani shrimp stored from 10 - 35°C showed that *Vibrio* species became dominant as spoilage progressed (Shamshad et al., 1990). At least five species of *Vibrio* are known decarboxylators of ornithine including *V. cholerae*, *V. mimicus*, *V. alginolyticus*, *V. parahaemolyticus*,

and *V. vulnificus* (McLaughlin, 1995). *Vibrio* species have been isolated as common microflora in wild and pond-raised shrimp (Vanderzant et al., 1970; Vanderzant et al., 1971; Christopher et al., 1978; Shamshad et al., 1990). Matches, (1982) found that *Proteus* species became dominant in Pacific shrimp as storage progressed at 16.7 and 22.2°C. This was associated with the production of indole. However, *P. mirabilis* and *P. morganii* (now called *Morganella morganii*) are also putrescine producers (Farmer, 1995). Thus, microorganisms like *Vibrio*, *Proteus*, and *Morganella* may be responsible for the production of putrescine in shrimp stored at temperatures of 10°C and above.

Shakila et al. (1995) showed limited increase, then a slight decrease of cadaverine levels in shrimp stored at 30°C, while, Mietz and Karmas (1978) reported a slightly lower value of 7.42 ppm for Class 3 shrimp relative to 7.80 ppm for Class 2 shrimp (Table 2). Nine species of *Vibrio* are active lysine decarboxylators, including, *V. cholera*, *V. mimicus*, *V. metschnikovii*, *V. cincinnatiensis*, *V. damsela*, *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus*, and *V. carchariae* (McLaughlin, 1995). Smith et al. (1984) isolated *Aeromonas* and *Proteus* in shrimp homogenates that were incubated above 10°C. *Aeromonas* species are known lysine decarboxylase positive microorganisms (Janda et al., 1995). It is apparent that the microflora is available for the production of cadaverine in shrimp decomposed at high temperatures. Thus, the lack of consistent cadaverine production in high temperature decomposed shrimp may be due to the limited availability of free lysine. Lysine generally makes up less than 1 % (molar %) of the free amino acid profile of shrimp (*Penaeus vannamei*) (Finne, 1992).

Hypotheses

1. Putrescine and/or cadaverine can be valuable and effective chemical indicators of decomposition in shrimp stored at temperatures ranging from 0-36°C.
2. Putrescine and/or cadaverine, as obtained in a single analysis, will provide equal or greater insight than indole as to the degree to which shrimp is decomposed.
3. The microflora responsible for the production of putrescine, cadaverine, and indole in shrimp will differ as a function of storage time and temperature.
4. Field testing of putrescine and cadaverine on actual commercial shrimp samples collected and organoleptically evaluated by FDA experts would verify the efficacy of putrescine and/or cadaverine as chemical indicators of shrimp decomposition.

Objectives

The objectives of this study are: (1) to determine the odor, indole, amine, and microbiological characteristics of Penaeid shrimp decomposed at temperatures ranging from 0-36°C, (2) to evaluate the efficacy of putrescine, cadaverine, and indole as chemical indicators of decomposition for Penaeid shrimp, (3) to determine the concentration(s) at which each chemical indicator confirms sensory decomposition, (4) to assess the feasibility of using putrescine/cadaverine analysis to supplement or improve the current decomposition evaluation system, (5) to determine the predominant microflora present and the microorganisms responsible for putrescine, cadaverine, and indole production throughout temperature dependent decomposition and (6) to verify the efficacy of putrescine, cadaverine, and indole as

shrimp decomposition indicators using actual commercial samples collected and organoleptically evaluated by FDA experts.

MATERIALS AND METHODS

Sample Procurement

In this study wild-caught domestic white shrimp (*Litopenaeus setiferus*) and pink shrimp (*L. brasiliensis*) and Nicaraguan aquacultured white shrimp (*L. vannamei*) were used for controlled decomposition experiments. These shrimp represent three different species acquired in different parts of the world from two different segments of the industry. The domestic wild-harvested shrimp were used initially because of ease in procurement and to establish general trends associated with shrimp decomposition. The Nicaraguan aquacultured shrimp were used for subsequent controlled decomposition experiments because the condition of the samples could be controlled and monitored from the time the shrimp were alive until termination of the experiments. Additionally, aquacultured shrimp represent a major portion of the shrimp currently produced in the world. A third set of commercial samples collected from around the world by FDA for decomposition analyses was subsequently incorporated into this study to verify data generated in the previous controlled decomposition experiments.

Domestic Wild Shrimp Procurement

The domestic shrimp used in this research were collected on two separate occasions from a commercial shrimp processor in Apalachicola, FL. The first batch, White shrimp (*Litopenaeus setiferus*), was harvested in January 1999 off the outer

banks of Apalachicola Bay, FL. The product, approximately 41 kg (90 lbs) of head-on shrimp, was delivered by the harvesting vessel to the processing plant in ice. Approximately 10 kg of this product was designated for this research and the remaining product was used for other experiments. Upon arrival at the plant, the shrimp were headed, rinsed, placed in 454-g (1-pint) plastic containers, and packed on ice in a 107-liter (113-quart) marine ice chest (Igloo® Corporation, Houston, TX) by plant and University of Florida personnel. The samples were then transported by air-conditioned van to the Aquatic Food Products Laboratory (AFPL) at the University of Florida in Gainesville, FL for decomposition at 0 and 12°C.

The second batch, Pink shrimp (*Litopenaeus brasiliensis*), was harvested from Apalachicola Bay in late March 1999. This product (approximately 5 kg (10 lbs) of head-on shrimp) was handled as stated above, packed as 225-250g increments in 1-pint (473-ml) Ziploc® freezer bags, and placed on ice in a 107-liter (113-quart) marine ice chest for transport to the AFPL. These samples were collected for decomposition at 24 and 36°C.

Nicaraguan Aquacultured Shrimp Procurement

Nicaraguan pond-raised shrimp (*Litopenaeus vannamei*) were collected for decomposition experiments from a commercial shrimp aquaculture and processing operation located in the Chinendega, Nicaragua area. The shrimp aquaculture facility was located on an island in the Gulf of Fonseca known as Isla Mangles Altos while the processing plant was in Chinendega. Transportation from the processing plant to the aquaculture facility consisted of a 1-hour, off-road truck ride followed by a 1-hour

trip by barge through the Estero Real, the estuary connecting the mainland to Isla Mangles Altos.

The water used to fill the ponds is pumped in from the estuary. The pond water temperature at this location was 28.7°C and had 30 ppt salinity. The ambient temperature during harvest was 25.3°C.

As is customary in the Central American shrimp aquaculture industry, the shrimp were harvested at night by a pond draining and capture method. With this method the pond water is allowed to drain into a canal and the shrimp are captured in a net that is positioned across the waterway outlet. During this process, the live shrimp is allowed to accumulate in the net just to the point of maximum manageable density before they are manually transferred to the rinse station. The rinse station consisted of a large vat of pond water in which 2 ft x 1 ft x 1 ft slotted plastic containers approximately $\frac{3}{4}$ full of shrimp were dipped and shaken to remove mud and debris from the product. After draining, the live rinsed shrimp were spread onto a culling table, separated from remaining debris, and immediately transferred to a tote containing ice slush in which the shrimp expired. Each tote held approximately 136 kg (300 lbs) of head-on shrimp mixed in a 1:1 ice slush to shrimp ratio. These totes were used to transport the shrimp from the pond location to the processing plant. Travel time from the pond to the processing plant was approximately 2 hours. The average head-on weight of the shrimp harvested from the pond was 12.3 g.

At the processing plant, one tote was allowed to sit for approximately 6 hours in anticipation of plant start-up operations. Upon start-up, the tote of shrimp and ice slush was drained and the head-on shrimp was placed on a stainless steel processing

table. The product was then rinsed and headed by plant personnel according to plant standard operating procedures and the headless shell-on product was placed in 710-ml (24-oz) sterile sample bags (Fisher Scientific, Atlanta, GA). Each sample bag contained approximately 225 g of shrimp and 100 bags were packed. The 100 sample bags of shrimp were then separated into 4 groups of 25 bags each and packed on ice with a 5-lb block of frozen shrimp in 4 45.4-l (48-qt) soft-sided, insulated coolers (California Innovations, Willowdale, Ontario, Canada). The samples were then packed as luggage and transported by air and van to the AFPL in Gainesville, FL. Decomposition experiments at 0, 12, 24, and 36°C began in the laboratory within 36 hours post-harvest.

Decomposition

Domestic Shrimp Decomposition

As was stated in the previous section, the shrimp samples (White shrimp-*L. setiferus*) collect for 0 and 12°C decomposition were packed in 454-g (1-pint) plastic containers and stored in ice for transport to the AFPL. Upon arrival at the laboratory, the samples of headless, shell-on shrimp designated for 0°C decomposition were left in the original containers, additional ice was added, and the 107-liter (113-quart) marine ice chest was placed in a 1.7°C (35°F) walk-in cooler for the duration of storage. Duplicate samples (2 454-g plastic containers) were collected on days 0, 4, 8, 12, and 16 for sensory, microbiological, and chemical analyses. The microbiological samples were set immediately after collection and the remaining shrimp from each storage increment were frozen at -28.9°C (-20°F) for subsequent

sensory and chemical analyses at the US Food and Drug Administration (FDA) Washington Seafood Laboratory (Washington, DC).

The shrimp designated for 12°C decomposition were stored in a 3.78-liter (1-gallon) plastic container on ice for 18 hours prior to use. For the 12°C decomposition experiment, approximately 450 g of the headless, shell-on shrimp were placed in 710-ml (24-oz) sterile sample bags (Fisher Scientific, Atlanta, GA) and stored at 12°C in a HotPack Refrigerator/Incubator (Philadelphia, PA). Duplicate samples were collected (2 450-g bags) on days 0, 1, 2, 3, and 4 for sensory, microbiological, and chemical analyses. Again, the microbiological samples were set immediately after collection and the remaining shrimp from each storage increment were frozen at -28.9°C (-20°F) for subsequent sensory and chemical analyses.

The shrimp samples (Pink shrimp-*P. brasiliensis*) collected for 24 and 36°C decomposition arrived at the AFPL packed as 20 225-g sample units in 1-pint (473-ml) Ziploc® freezer bags stored on ice. Upon arrival at the laboratory, the sample bags were removed from the ice, equilibrated to approximately room temperature using running tap water, and then stored at 24 and 36°C in HotPack Refrigerator/Incubators for decomposition. Equilibration to room temperature was done to simulate product temperature immediately post-harvest. The running tap water was allowed no direct contact with the shrimp. Two 225-g sample bags decomposed at 24°C were collected on hours 0, 12, 24, 36, and 48 while 2 225-g sample bags (duplicate samples) decomposed at 36°C were collected on hours 0, 6, 12, 18, and 24 for sensory, microbiological, and chemical analyses. After collection, samples were handled as stated previously.

Sample bags of shrimp frozen at -28.9°C (-20°F) were packed with approximately 4.5kg (10lbs) of dry ice in Styrofoam insulated cardboard shipping boxes. Each shipping box was stuffed with newspaper, sealed with packing tape, and shipped for overnight delivery.

Nicaraguan Shrimp Decomposition

Upon arrival at the laboratory, the 225-g bags of aquacultured, headless, shell-on shrimp designated for 0°C decomposition were removed from the transportation coolers, repacked on ice in a 114-liter (120-quart) marine ice chest, and placed in a 1.7°C (35°F) walk-in cooler for the duration of storage. The shrimp samples designated for 12, 24, and 36°C decomposition were removed from the transportation coolers, equilibrated to approximately room temperature using running tap water, and then stored at the appropriate temperatures in refrigerator/incubators for decomposition. The samples were equilibrated to approximately room temperature to simulate pond water temperature at the time of harvest. The running tap water was allowed no direct contact with the shrimp.

Placement of the individual 710-ml sample bags (each containing approximately 225g of headless, shell-on shrimp) within each refrigerator/incubator was set-up to allow for the most even temperature distribution reasonably possible inside the refrigerator/incubators. To accomplish this, the sample bags were spread out evenly (no overlap) across the middle three of five total shelves located inside each refrigerator/incubator. The temperature inside each refrigerator/incubator was monitored using the external digital display and with a digital thermometer (Fisher

Scientific, Atlanta, GA) immersed in water located on the middle shelf of each refrigerator/incubator.

At each decomposition temperature, triplicate sample bags of shrimp were removed from the refrigerator/incubators at pre-determined time intervals and at intervals encompassing the transition from acceptable to decomposed shrimp. The transition intervals were determined by sensory analyses. Shrimp samples were collected on days 0, 4, 8, 12, 14, 16, 17, 18, and 20 and on days 0, 1, 2, 2.5, 3, 3.5, 4, and 4.5 during the 0 and 12°C decomposition, respectively. Additionally, shrimp samples were collected on hours 0, 4, 8, 10, 12, 14, 16, 20, and 24 and on hours 0, 2, 4, 6, 8, 10, and 12 during the 24 and 36°C decomposition.

Upon removal from the refrigerator/incubators, duplicate 25-g subsamples of shrimp were taken from each sample bag and immediately prepared for microbiological analysis. At the 12, 24, and 36°C decomposition temperatures, the remainder of the shrimp in each bag was submersed in ice slush for 3-5 minutes to minimize further decomposition and then frozen at -28.9°C (-20°F) for subsequent sensory and chemical analyses at the FDA Northeast Regional Seafood Laboratory (Jamaica, NY) and the FDA Washington Seafood Laboratory (Washington, DC), respectively. No ice slush treatment was needed for the 0°C decomposition samples since the shrimp had been stored on ice.

Sample bags of shrimp frozen at -28.9°C (-20°F) were packed with approximately 4.5kg (10lbs) of dry ice in Styrofoam insulated cardboard shipping boxes. Each shipping box was stuffed with newspaper, sealed with packing tape, and shipped for overnight delivery.

Microbiological Analyses

Aerobic Plate Counts

At each sample time for each storage temperature, duplicate (domestic shrimp) or triplicate (Nicaraguan shrimp) samples (25g each) of shrimp were randomly selected and blended with 225 ml of 0.1% sterile peptone diluent (Difco, Detroit, MI) in a sterile 454-ml (1-pint) Ball Mason Jar (Alltrista Corporation, Muncie, IN) for 2 minutes at low speed (approximately 8,000 rpm) (Messer et al., 1992). Blender blades were sterilized by alcohol flaming prior to blending. One ml (divided over four plates), 0.1 ml, and 0.1 ml volumes of appropriate serial dilutions (0.1% sterile peptone) of each sample homogenate were surface plated on Tryptic Soy Agar (TSA, Difco, Detroit, MI). Inoculated plates were incubated at 23-25°C for 72 hours.

Microbial Type Characterizations

The following procedures were used to produce a bacterial type characterization for samples of shrimp decomposed at different time/temperature combinations. One to three representatives of each colony type appearing on a representative countable plate at each designated time/temperature combination were picked and transferred to TSA slants (Vanderzant et al., 1985). These slants were incubated for 48 hr at 23-25°C or until a healthy bacterial culture could be seen on the slant. The identity of each isolate was determined by diagnostic tests and schemes as described by Vanderzant and Nickelson (1969) and Holt (1984, 1986), in addition to the utilization of an automated bacterial identification system (Vitek Jr., Vitek Systems, Hazelwood, MO). Manufacturers instructions were followed when

identifying bacterial isolates using the Vitek Jr. Each colony was identified to the generic and species level (where possible) and expressed as a percentage of the total number of colonies appearing on countable plates. Microbial type characterizations were determined for all decomposition time/temperature combinations on the domestic shrimp. For the Nicaraguan shrimp, microbial type characterizations were determined for time intervals representing the beginning, middle, and end of the decomposition time at each temperature. The decision to reduce the number of microbial type characterizations determined for the Nicaraguan shrimp was made to decrease the total number of bacterial isolates identified while still acquiring adequate data relating to microbial flora changes as a function of time and temperature of decomposition. Microbial type characterizations on Nicaraguan shrimp decomposed at 0 and 12°C were determined for days 0, 8, 16, and 20 and 0, 2, and 4, respectively. At decomposition temperatures of 24 and 36°C, microbial type characterizations were determined on shrimp decomposed for 0, 12, and 24 and 0, 6, and 12 hours, respectively.

Microbial Production of Putrescine, Cadaverine, and Indole

All microorganisms isolated from the domestic and Nicaraguan shrimp were tested for their ability to produce putrescine from ornithine, cadaverine from lysine, and indole from tryptophan. Putrescine and cadaverine production was determined using Bacto® Decarboxylase Base Moeller Medium with the addition of 10g of L-ornithine or L-lysine, respectively. This medium was prepared and used according to manufacturers instructions with the exception that incubation of the isolates was conducted at 23-25°C in addition to 35 ± 2 °C (Difco, 1998). Indole production by

each bacterial isolate was determined using BBL™ DrySlide™ Indole test. This test was performed according to manufacturers instructions (Becton Dickinson Microbiology Systems, Sparks, MD).

Sensory Analyses

Sensory Analyses of Domestic Shrimp

Frozen headless, shell-on domestic shrimp were analyzed in duplicate for degree of decomposition at the FDA Washington Seafood Laboratory by one FDA seafood sensory expert and one University of Florida seafood sensory analyst. These samples were evaluated according to standard FDA procedures, which progressed as follows. Individual frozen shrimp sample bags were thawed under cool running water. Once the shrimp were thawed, each bag of shrimp was emptied onto a stainless steel tray and the shrimp were spread out for visual evaluation. Then each individual shrimp was broken near the center of the tail and analyzed for odors of decomposition. After each shrimp had been evaluated the bag of shrimp was assigned a decomposition rating based on the FDA three-class system. Class 1 shrimp possess very fresh odors or other odors characteristic of the product that are not identifiable as decomposition. Class 2 includes shrimp with a slight odor of decomposition. Class 3 shrimp possess the distinct and unmistakable odors of decomposition (Code of Federal Regulations, 1991). Once each duplicate bag of shrimp had been evaluated, a decomposition rating was assigned to the shrimp decomposed at each time/temperature combination as a whole.

Sensory Analyses of Nicaraguan Shrimp

Frozen headless, shell-on Nicaraguan shrimp were analyzed in triplicate for degree of decomposition at the FDA Northeast Regional Seafood Laboratory by three FDA seafood sensory experts and one University of Florida seafood sensory analyst. Procedures followed for these evaluations were the same as stated above except that the analysts assigned the shrimp to “unofficial” classifications that were more descriptive than the three-class system. The classifications used were Pass (P1), Low Quality Pass (LQP), Borderline Pass (BLP), Borderline Fail (BLF), Fail Class 2 (F2), Fail Class 2 Advanced (F2Adv), and Fail Class 3 (F3). Relative to the three-class system described above, P1, LQP, and BLP fall into the Class 1 category, BLF, F2, and F2Adv are part of the Class 2 category, and F3 is the same as Class 3 shrimp. For graphical purposes a F2+ category was created to represent F2, F2Adv, and F3 decomposed shrimp. These classifications essentially breakdown Class1 and Class 2 decomposition of shrimp into more specific categories. This was done to allow for acquisition of more precise data relative to the degree of decomposition of the shrimp in the transition area from acceptable (Class 1) to unacceptable (Class 2) shrimp.

Chemical Analyses

Putrescine and Cadaverine Analyses

Chemical analyses of domestic and Nicaraguan shrimp samples for levels of putrescine and cadaverine were conducted at the FDA Washington Seafood Laboratory. Triplicate samples of shrimp decomposed at all time temperature combinations were analyzed for putrescine and cadaverine concentrations according to the Association of Official Analytical Chemists (AOAC) Official Method 996.07

as modified by FDA for shrimp (AOAC, 1999). In collaborative study, the unmodified method produced repeatability relative standard deviations of 5.2 and 15% and reproducibility relative standard deviations of 8.8 and 18% for putrescine and cadaverine, respectively (Rogers and Staruskiewicz, 1997). Additionally, this method is sensitive for putrescine and cadaverine detection at ≥ 0.3 and ≥ 0.5 ppm, respectively (AOAC, 1998). Modifications from Official Method 996.07 included the following: 1) modified extraction solvent – 75% Methanol/25% 0.4 N HCl; 0.5% KCl, 2) derivatization volume reduced to 5ml, 3) use 75% methanol (no salt) during evaporation, 4) use 50% ethyl acetate in toluene for solid-phase extraction (SPE) step (Staruskiewicz and Rogers, Personal Communication, 2000).

For the domestic shrimp samples triplication was accomplished by compositing duplicate sample bags from each time/temperature combination into a single batch, homogenizing the batch, and pulling three 10-g samples from the homogenized batch for analysis. In the case of the Nicaraguan shrimp, triplicate sample bags were homogenized and analyzed individually to achieve triplication.

Indole Analyses

Analyses of domestic and Nicaraguan shrimp for indole levels were also conducted at the FDA Washington Seafood Laboratory. Triplicate samples of shrimp decomposed at all time temperature combinations were analyzed for indole concentrations according to the AOAC Official Method 981.07 (AOAC, 1999). This method is sensitive for indole detection at $>1 \mu\text{g}/100\text{g}$. In collaborative study, AOAC Official Method 981.07 produced repeatability and reproducibility coefficients of variation of 5.6 and 11%, respectively for samples with indole levels $>20\mu\text{g}/100\text{g}$

(Chambers and Staruszkiewicz, 1981). The description of sample triplication stated above also applies to the indole analyses.

Reported Units

Indole is reported by the FDA in $\mu\text{g}/100\text{g}$ or parts per one hundred million (100,000,000) with a minimum detection level of $>1 \mu\text{g}/100\text{g}$ as shown in AOAC Official Method 981.07 (AOAC, 1999). Also, previous regulatory policy for decomposed shrimp was based on indole concentrations of $25 \mu\text{g}/100\text{g}$ or greater (FDA, 1996a). Current methodology for detection of putrescine and cadaverine (AOAC Official Method 996.07) shows that putrescine and cadaverine are reported in $\mu\text{g}/\text{g}$ or parts per million (ppm) with minimum detection levels of ≥ 0.3 and ≥ 0.5 ppm, respectively (AOAC, 1999). For comparative purposes it should be noted that units of $\mu\text{g}/100\text{g}$ are two orders of magnitude or 100 times smaller than $\mu\text{g}/\text{g}$ or parts per million (ppm) when similar significant digits are used. In relative terms this means that by current methodology, indole is detected in 100 times smaller quantities than putrescine and cadaverine. Reporting indole concentrations in $\mu\text{g}/100\text{g}$ and putrescine and cadaverine concentrations in parts per million (ppm) facilitates graphical presentation of the data by allowing the data to be shown on a common scale and in values with relative similarity. Reporting the data in this form also adheres to traditional values and units used for regulatory policy and reported in the literature. Therefore, indole was reported in $\mu\text{g}/100\text{g}$ and putrescine and cadaverine were reported in parts per million (ppm) in this dissertation.

FDA Commercial Sample Analyses

Commercial shrimp samples collected and analyzed for decomposition by the FDA Pacific Regional Laboratory (Bothell, WA) over a period of approximately 6 months were retained for chemical analyses. The frozen samples were packed with dry ice in insulated cardboard shipping boxes and shipped to the FDA Washington Seafood Laboratory and stored frozen ($-28.9^{\circ}\text{C}/-20^{\circ}\text{F}$) until analyzed. At the FDA Washington Seafood Laboratory, the samples were unpacked, the sensory classifications were recorded, and analyses for levels of putrescine, cadaverine, and indole were conducted as stated above.

Statistical Analyses

Statistical analyses of the data compiled during the Nicaraguan shrimp decomposition experiments began by fitting nonlinear regression curves based on Mitcherlich's equation to logarithmically transformed putrescine, cadaverine, and indole concentrations at each temperature of decomposition (Myers, 1986). Logarithmic transformations were made to produce better curve fitting results, especially on data sets with a large number of negligible or small values (<1 ppm) followed by a relative few number of large values (>300 ppm).

Mitcherlich's equation is written as $y = \alpha + \beta e^{\gamma x}$, where y is putrescine, cadaverine, or indole, x is time, and α , β , and γ are the parameters associated with this model. This equation is an exponential equation that is useful in agriculture and chemistry when the variable of interest (i.e. putrescine, cadaverine, or indole) increases exponentially with time (Myers, 1986). Using Mitcherlich's equation, the concentration of a selected chemical indicator can be predicted relative to temperature

and time. These curves were fit using the NLIN procedure in SAS STAT. The results from this nonlinear regression analysis were used to help determine the threshold concentrations at which each chemical indicator would confirm sensory decomposition

Contingency table analyses comparing putrescine, cadaverine, and indole at the selected threshold levels relative to the FDA sensory classification (Pass vs. Fail) of the shrimp samples was conducted. Contingency tables were constructed to test the efficacy of selected levels of putrescine, cadaverine, and indole as chemical indicators of decomposition in shrimp. Measures for sensitivity, specificity, and predictive value were then calculated based on contingency table values and correlation between the FDA sensory classifications and selected concentrations of putrescine, cadaverine, and indole was tested using the Kendall Partial Rank Correlation Coefficient (Kendall's Tau) (Siegel, 1956).

Sensitivity measures the probability that the chemical indicator at the selected level will predict (confirm) decomposition when the sample fails sensory analysis while specificity measures the probability that the chemical indicator at the selected level will not predict decomposition when the sample passes sensory analysis. Predictive value measures the probability that the sample will fail sensory analysis when the chemical indicator predicts decomposition. Finally, Kendall's Tau (The Kendall Partial Rank Correlation Coefficient) is a measure of the correlation between FDA sensory classifications and the selected concentrations of putrescine, cadaverine, and indole (Siegel, 1956).

Contingency table analyses, measures for sensitivity, specificity, and predictive value, and the Kendall Partial Rank Correlation Coefficient (Kendall's Tau) were also conducted for commercial shrimp samples collected by the FDA Pacific Regional Laboratory (Bothell, WA).

RESULTS AND DISCUSSION

Domestic Shrimp Decomposition

Microbiological Analyses

Mean aerobic plate counts (APC- $\log_{10}/g$) and microbial type characterizations for wild domestic shrimp (*Litopenaeus setiferus*) decomposed at 0°C for 16 days and 12°C for 4 days are shown in Table 3. The APCs ($\log_{10}/g$) of the shrimp increased as expected over the 16 and 4-day storage periods. Mean APCs ($\log_{10}/g$) increased from 4.6 to 8.3 and 4.5 to 8.8 for the 0 and 12°C decomposed shrimp, respectively. These results generally agree with previous research on wild domestic shrimp stored at similar temperatures. Benner et al. (1994) reported APCs ($\log_{10}/g$) increasing from 3.9 to 8.8 for Gulf of Mexico brown shrimp (*Penaeus aztecus*) stored on ice for 16 days. Additionally, Gulf Coast shrimp stored in crushed ice for 16 days were shown to have APCs ($\log_{10}/g$) increasing from 4.1 to 7.5 (Campbell and Williams, 1952). Chang et al. (1983) reported APCs ($\log_{10}/g$) of Gulf white (*P. setiferus*) and pink (*P. duorarum*) shrimp stored at 12°C for 3 days increasing from approximately 5.0 to 8.4.

The microbial type characterization of domestic shrimp stored at 0°C showed a microflora initially dominated by coryneform bacteria and *Moraxella* with lower levels of *Bacillus*, *Lactobacillus*, *Micrococcus*, and *Pseudomonas* detected. On storage day 8 the levels of coryneform bacteria and *Moraxella* had decreased, the levels of *Lactobacillus* and *Pseudomonas* had increased, and *S. putrefaciens*

represented 20% of the microflora isolated. By day 16 *Shewanella putrefaciens* was the dominant microbial type isolated with *Pseudomonas* and *Moraxella* representing the majority of the remaining microflora. These results generally agree with previous studies on wild-caught Penaeid shrimp stored at 0°C with the exception that in these investigations *S. putrefaciens* was included in the *Pseudomonas* or *Alteromonas* category (Benner et al., 1994; Shamshad et al., 1990). As was mentioned in the literature review, *S. putrefaciens* was previously named *P. putrefaciens* and *A. putrefaciens* (MacDonell and Colwell, 1985).

The microbial type characterization of domestic shrimp stored at 12°C also showed microflora initially dominated by coryneform bacteria and *Moraxella*, however, lower levels of *Proteus mirabilis* and *S. putrefaciens* were isolated. On day 2 the microflora was dominated by *S. putrefaciens* with *Moraxella*, *Aeromonas*, and coryneform bacteria representing the majority of the remaining microbial isolates. Lower levels of *Micrococcus* and *Pseudomonas* were also detected. By day 4 the microflora was dominated by *Moraxella* and *S. putrefaciens* while coryneform bacteria and *Alcaligenes* comprised the remaining microflora. Shamshad et al. (1990) reported similar results on *P. merguensis* stored at 10°C for 4 days.

Mean APCs ($\log_{10}/g$) and microbial type characterizations for wild domestic shrimp (*Litopenaeus brasiliensis*) decomposed at 24°C for 48 hours and 36°C for 24 hours are shown in Table 4. The APCs ($\log_{10}/g$) of the shrimp increased as expected over the 48 and 24-hour storage periods. Mean APCs ($\log_{10}/g$) increased from 5.5 to 9.7 and 5.3 to 8.8 for the 24 and 36°C decomposed shrimp, respectively. These results also generally agree with previous research on wild domestic shrimp stored at

similar temperatures. APCs ($\log_{10}/g$) increasing from approximately 5.0 to 9.4 have been reported for Gulf shrimp (*P. setiferus* and *P. duorarum*) stored at 22°C for 24 hours (Chang et al., 1983). Cobb et al. (1977) published APCs ($\log_{10}/g$) increasing from approximately 4.1 to 8.3 for *P. setiferus* stored at 37°C for 24 hours. Additionally, Shamshad et al. (1990) reported APCs ($\log_{10}/g$) for wild Pakistani *Penaeus merguensis* stored at 25 and 35°C for 24 hours increasing from 5.7 to 9.5 and 5.7 to 9.8, respectively.

The microbial type characterization of domestic shrimp stored at 24°C showed an initial microflora dominated by coryneform bacteria, *Comamonas*, and *Pseudomonas* with lower levels of *Flavobacterium odoratum*, *S. putrefaciens*, and *Acinetobacter* detected. At hour 24 *F. odoratum* and coryneform bacteria comprised the majority of the microbial isolates with lower levels of *Acinetobacter*, *Morganella morganii*, *S. putrefaciens*, *Aeromonas*, *Citrobacter*, *Proteus vulgaris*, and *Bacillus* detected. By hour 48 the microflora was dominated by *F. odoratum* and *M. morganii* with lower levels of *P. vulgaris*, *Pasteurella haemolytica*, *Bacillus*, coryneform bacteria, and *Lactobacillus* isolated. These results are similar to those of Matches (1982) who showed a microflora initially dominated by *Moraxella* and coryneform bacteria that shifted to a microflora dominated by *Proteus* spp. in *Pandalus jordani* stored at 22.2°C for 2 days. It should be noted that the *M. morganii* isolated in our study would have been classified as *Proteus* spp. in Matches (1982) since *Morganella morganii* was formerly known as *Proteus morganii* (Gilchrist, 1995).

The microbial type characterization of domestic shrimp stored at 36°C showed an initial microflora dominated by coryneform bacteria, *Pseudomonas*, and

Stenotrophomonas maltophilia with lower levels of *Acinetobacter*, *Comamonas*, *P. vulgaris*, *Vibrio parahaemolyticus*, *S. putrefaciens*, and unidentified bacteria detected. At hour 12 *V. parahaemolyticus*, *Aeromonas*, *M. organii*, and *Photobacterium damsela* were prevalent with lower levels of *Staphylococcus*, *P. haemolytica*, *P. vulgaris*, and *S. putrefaciens* isolated. By hour 24 a relatively even distribution of microbial types was isolated with no individual bacterial type representing more than 20% of the total microflora. *P. vulgaris*, *Alcaligenes*, *Bacillus*, *M. organii*, *V. alginolyticus*, coryneform bacteria, and unidentified bacteria each represented greater than or equal to 10% of the total microflora while *Lactobacillus*, *S. putrefaciens*, *Eikenella*, and *Pseudomonas* each comprised less than 5% of the total microflora. These results differ somewhat for Shamshad et al. (1990) who reported a microflora initially dominated by *Moraxella*, *Pseudomonas*, and *Micrococcus* which shifted to a microflora dominated by *Vibrio* spp. for shrimp decomposed at 35°C. Our results are however, consistent with what would be expected for shrimp decomposed at 36°C since the majority of organisms isolated were mesophilic in nature.

The abilities of bacteria isolated from wild domestic shrimp (*L. setiferus* and *L. brasiliensis*) decomposed at 0, 12, 24, and 36°C to produce putrescine from ornithine, cadaverine from lysine, and indole from tryptophan are listed in Tables 5-8. A total of 254 bacteria were isolated and identified. Bacteria capable of producing putrescine from ornithine were *S. putrefaciens* (5) and *P. fluorescens* (1) isolated from shrimp decomposed at 0°C, *S. putrefaciens* (7) and *P. mirabilis* (2) isolated from shrimp decomposed at 12°C, *M. organii* (5), *S. putrefaciens* (2), *Aeromonas* (2), and *V. alginolyticus* (1) isolated from shrimp decomposed at 24°C, and *M.*

morganii (6), *S. putrefaciens* (2), *V. alginolyticus* (2), *V. parahaemolyticus* (2), *Aeromonas* (1), *Citrobacter braaki* (1), and *P. mirabilis* (1) isolated from shrimp decomposed at 36°C.

Microorganisms capable of producing cadaverine from lysine were *Pseudomonas* spp. (2), *P. fluorescens* (1), and *P. fluorescens/putida* (1) isolated from shrimp decomposed at 0°C, *Pseudomonas* spp. (1) and *P. mirabilis* (1) isolated from shrimp decomposed at 12°C, *Comamonas testeroni* (1) isolated from shrimp decomposed at 24°C, and *V. alginolyticus* (2), *C. testeroni* (1), *P. damsela* (1), and *V. parahaemolyticus* (1) isolated from shrimp decomposed at 36°C.

No microorganisms isolated from shrimp decomposed at 0°C produced indole from tryptophan. Bacteria capable of producing indole from tryptophan were *Lactobacillus* (1) isolated from shrimp decomposed at 12°C, *P. vulgaris* (7), *M. morganii* (5), *Aeromonas* (2), *F. odoratum* (1), and *V. alginolyticus* (1) isolated from shrimp decomposed at 24°C, and *M. morganii* (5), *P. vulgaris* (4), *Aeromonas* (3), *V. alginolyticus* (2), *V. parahaemolyticus* (2), and *Bacillus* (1) isolated from shrimp decomposed at 36°C.

The data listed in Tables 5-8 indicate that *S. putrefaciens* was the major microorganism responsible for putrescine production in the shrimp decomposed at 0 and 12°C while *M. morganii*, *Vibrio* spp., *S. putrefaciens*, and *Aeromonas* were responsible for putrescine production in shrimp decomposed at 24 and 36°C. This data generally agrees with previous research conducted on shrimp decomposed at similar temperatures in that psychrotrophic microorganisms (i.e., *Pseudomonas* and *Shewanella*) tend to dominate the microflora of shrimp decomposed at lower

temperatures while mesophilic microorganisms (i.e., *M. morganii*, *Vibrio* spp., and *Aeromonas*) become more prevalent in shrimp decomposed at higher temperatures (Matches, 1982; Vanderzant et al., 1973; Shamshad et al., 1990).

The data listed in Tables 5-8 also indicate that no single organism or group of organisms isolated was dominantly responsible for potential indole production and *Pseudomonas* spp. were responsible for cadaverine production in shrimp decomposed at 0 and 12°C. *Vibrio* spp. (*V. alginolyticus* and *V. parahaemolyticus*) were clearly the dominant cadaverine producers with *Proteus vulgaris* and *M. morganii* present as the dominant indole producers on shrimp decomposed at 24 and 36°C. *Vibrio* spp. and *Aeromonas* were isolated as less prevalent indole producers on shrimp decomposed at 24 and 36°C. As previously stated, we see that the mesophilic microorganisms dominated the microflora at higher decomposition temperatures.

The above results showing putrescine production by *S. putrefaciens* isolated from shrimp decomposed at 0 and 12°C and putrescine production by *M. morganii*, *Vibrio* spp., *S. putrefaciens*, and *Aeromonas* isolated from shrimp decomposed at 24 and 36°C indicate the microbiological potential for putrescine to be an effective chemical indicator of decomposition for shrimp decomposed over a wide temperature range. The production of cadaverine by *Pseudomonas* spp. isolated from shrimp decomposed at 0 and 12°C and cadaverine production by *Vibrio* spp. isolated from shrimp decomposed 24 and 36°C also show the microbiological potential for cadaverine to be an effective chemical indicator of decomposition for shrimp decomposed over a wide temperature range. A lack of dominant indole producing isolates in shrimp decomposed at 0 and 12°C indicate that microbiological limitations

may hinder the effectiveness of indole as a chemical indicator in shrimp decomposed at lower temperatures. However, the prevalence of indole producing *P. vulgaris*, *M. morganii*, *Vibrio* spp. and *Aeromonas* isolated from shrimp decomposed at 24 and 36°C indicate the microbiological potential for indole to be effective chemical indicators of shrimp decomposed at higher temperatures.

Table 3 - Decomposition ratings, mean putrescine, cadaverine, indole levels, aerobic plate counts ($\log_{10}/g$), and percent characterization of microbial types for domestic Penaeid shrimp (*L. setiferus*) stored at 0 and 12°C^{a,b}

Decomposition Rating	0°C					12°C				
	Day 0	Day 4	Day 8	Day 12	Day 16	Day 0	Day 1	Day 2	Day 3	Day 4
	Class 1	Class 1	Class 1	Class 1 LQ	Class 2	Class 1	Class 1 LQ	Class 2	Class 2 Adv	Class 3
Putrescine ppm	1.4 ± 0.6	1.0 ± 0.4	1.1 ± 0.5	16.5 ± 1.2	77.8 ± 10.0	1.4 ± 0.6	12.2 ± 1.2	140.2 ± 1.6	230.7 ± 13.9	613.5 ± 14.8
Cadaverine ppm	0 ± 0	0 ± 0	0 ± 0	0 ± 0	2.2 ± 0.8	0 ± 0	1.3 ± 0.3	2.5 ± 0.4	4.2 ± 0.2	6.0 ± 0.7
Indole $\mu g/100g$	0 ± 0	0 ± 0	0 ± 0	5.2 ± 1.1	21.2 ± 1.1	0 ± 0	3.4 ± 1.2	12.0 ± 1.5	51.4 ± 0.9	114.1 ± 1.6
APC($\log_{10}$)	4.6	4.7	5.4	7.6	8.3	4.5	5.4	7.8	8.5	8.8
Organism	Percent Distribution of Microbial Types									
<i>Acinetobacter</i>							6			
<i>Aeromonas</i>								14		
<i>Alcaligenes</i>										12
<i>Bacillus</i>	5									
<i>coryneform</i>	44	7		2	1	49	14	12	16	14
<i>Flavobacterium</i>		10								
<i>Lactobacillus</i>	1	17	20	11	2		12		6 (I)	
<i>Micrococcus</i>	2	6					5			
<i>Moraxella</i>	44	50	32	29	27	35 13 (P,C)	22	16	13	41
<i>P. mirabilis</i>								7 (P)		
<i>Pseudomonas</i>	4	7 (P,C)	28	42 (C)	28 (C)	3 (P)	3 (C)	5	16	
<i>S. putrefaciens</i>		3 (P)	20 (P)	16 (P)	42 (P)	3 (P)	38 (P)	46 (P)	49 (P)	33 (P)

^aThe letters in parentheses indicate microbial isolates capable of producing putrescine (P), cadaverine (C), and indole (I).

^bNumbers following the $\pm$ sign are the standard deviations of the means of putrescine (ppm), cadaverine (ppm), and indole ($\mu g/100g$) listed in each cell.

Table 4 - continued

Organism	24°C					36°C				
	Hour 0	Hour 12	Hour 24	Hour 36	Hour 48	Percent Distribution of Microbial Types				
						Hour 0	Hour 6	Hour 12	Hour 18	Hour 24
<i>Ph. damsela</i>							12	15 (C)		
<i>P. mirabilis</i>									6 (P)	
<i>P. vulgaris</i>			1 (I)	8 (I)	6 (I)	5 (I)		3 (I)	6 (I)	19 (I)
<i>Pseudomonas</i>	18					19	20			2
<i>S. putrefaciens</i>	5 (P)		6 (P)			2		3	23 (P)	3 (P)
<i>Staphylococcus</i>								5		
<i>S. maltophilia</i>						14				
<i>V. alginolyticus</i>				5 (P,I)			2		37 (P,C,I)	11 (P,C,I)
<i>V. parahaemolyticus</i>						5 (P,I)		28 (P,C,I)		
Unidentified				2		8	2			11

^aThe letters in parentheses indicate microbial isolates capable of producing putrescine (P), cadaverine (C), and indole (I).

^bNumbers following the ± sign are the standard deviations of the means of putrescine (ppm), cadaverine (ppm), and indole (µg/100g) listed in each cell.

Table 5 - Production of putrescine, cadaverine, and indole by bacteria isolated from domestic shrimp decomposed at 0°C

Isolate Number	Days of Storage	Organism	Putrescine	Cadaverine	Indole
0-1	0	coryneform	-	-	-
0-2	0	<i>Micrococcus</i>	-	-	-
0-3	0	coryneform	-	-	-
0-4	0	coryneform	-	-	-
0-5	0	<i>Acinetobacter</i>	-	-	-
0-6	0	coryneform	-	-	-
0-7	0	coryneform	-	-	-
0-8	0	<i>Moraxella</i>	-	-	-
0-9	0	coryneform	-	-	-
0-10	0	coryneform	-	-	-
0-11	0	<i>Moraxella</i>	-	-	-
0-12	0	coryneform	-	-	-
0-13	0	coryneform	-	-	-
0-14	0	<i>Pseudomonas fl/put</i>	-	-	-
0-15	0	<i>Moraxella</i>	-	-	-
0-16	0	coryneform	-	-	-
0-17	0	coryneform	-	-	-
0-18	0	<i>Bacillus</i>	-	-	-
0-19	0	<i>Micrococcus</i>	-	-	-
0-20	0	coryneform	-	-	-
0-21	0	<i>Moraxella</i>	-	-	-
0-22	0	<i>Lactobacillus</i>	-	-	-
0-23	0	coryneform	-	-	-
0-24	0	<i>Moraxella</i>	-	-	-
0-25	0	<i>Micrococcus</i>	-	-	-
0-26	4	<i>P. fluorescens</i>	+	-	-
0-27	4	<i>Moraxella</i>	-	-	-
0-28	4	<i>Moraxella</i>	-	-	-
0-29	4	<i>Moraxella</i>	-	-	-
0-30	4	<i>Pseudomonas</i>	-	-	-
0-31	4	<i>Flavobacterium</i>	-	-	-
0-32	4	<i>S. putrefaciens</i>	+	-	-
0-33	4	<i>Micrococcus</i>	-	-	-
0-34	4	coryneform	-	-	-
0-35	4	<i>Moraxella</i>	-	-	-
0-36	4	coryneform	-	-	-
0-37	4	<i>P. fluorescens</i>	-	+	-
0-38	4	<i>Micrococcus</i>	-	-	-
0-39	4	<i>Lactobacillus</i>	-	-	-
0-40	4	<i>Micrococcus</i>	-	-	-

Table 5 - continued

Isolate Number	Days of Storage	Organism	Putrescine	Cadaverine	Indole
0-41	4	<i>F. odoratum</i>	-	-	-
0-42	4	<i>Moraxella</i>	-	-	-
0-43	8	<i>S. putrefaciens</i>	-	-	-
0-44	8	<i>S. putrefaciens</i>	+	-	-
0-45	8	<i>Pseudomonas</i>	-	-	-
0-46	8	<i>Moraxella</i>	-	-	-
0-47	8	<i>Moraxella</i>	-	-	-
0-48	8	<i>Moraxella</i>	-	-	-
0-49	8	<i>Moraxella</i>	-	-	-
0-50	8	<i>Moraxella</i>	-	-	-
0-51	8	<i>Lactobacillus</i>	-	-	-
0-52	8	<i>Lactobacillus</i>	-	-	-
0-53	12	<i>S. putrefaciens</i>	+	-	-
0-54	12	<i>Pseudomonas</i>	-	+	-
0-55	12	coryneform	-	-	-
0-56	12	<i>Moraxella</i>	-	-	-
0-57	12	<i>Moraxella</i>	-	-	-
0-58	12	<i>Lactobacillus</i>	-	-	-
0-59	12	<i>Moraxella</i>	-	-	-
0-60	12	<i>Moraxella</i>	-	-	-
0-61	12	<i>Lactobacillus</i>	-	-	-
0-62	12	<i>Moraxella</i>	-	-	-
0-63	12	<i>Moraxella</i>	-	-	-
0-64	16	<i>Pseudomonas</i>	-	-	-
0-65	16	coryneform	-	-	-
0-66	16	<i>Pseudomonas fl/put</i>	-	+	-
0-67	16	<i>S. putrefaciens</i>	+	-	-
0-68	16	<i>S. putrefaciens</i>	+	-	-
0-69	16	<i>P. fluorescens</i>	-	-	-
0-70	16	<i>Pseudomonas</i>	-	+	-
0-71	16	<i>Moraxella</i>	-	-	-
0-72	16	<i>Pseudomonas</i>	-	-	-
0-73	16	<i>Moraxella</i>	-	-	-
0-74	16	<i>Lactobacillus</i>	-	-	-

Table 6 - Production of putrescine, cadaverine, and indole by bacteria isolated from domestic shrimp decomposed at 12°C

Isolate Number	Days of Storage	Organism	Putrescine	Cadaverine	Indole
12-1	0	coryneform	-	-	-
12-2	0	<i>Moraxella</i>	-	-	-
12-3	0	<i>S. putrefaciens</i>	+	-	-
12-4	0	<i>Moraxella</i>	-	-	-
12-5	0	coryneform	-	-	-
12-6	0	<i>P. mirabilis</i>	+	+	-
12-7	0	coryneform	-	-	-
12-8	0	coryneform	-	-	-
12-9	0	coryneform	-	-	-
12-10	0	coryneform	-	-	-
12-11	1	coryneform	-	-	-
12-12	1	<i>S. putrefaciens</i>	+	-	-
12-13	1	<i>S. putrefaciens</i>	+	-	-
12-14	1	coryneform	-	-	-
12-15	1	<i>S. putrefaciens</i>	+	-	-
12-16	1	coryneform	-	-	-
12-17	1	<i>Moraxella</i>	-	-	-
12-18	1	<i>Pseudomonas</i>	-	+	-
12-19	1	<i>Micrococcus</i>	-	-	-
12-20	1	<i>Acinetobacter</i>	-	-	-
12-21	1	<i>Moraxella</i>	-	-	-
12-22	1	<i>Lactobacillus</i>	-	-	-
12-23	1	<i>Lactobacillus</i>	-	-	-
12-24	1	<i>Moraxella</i>	-	-	-
12-25	2	<i>S. putrefaciens</i>	+	-	-
12-26	2	<i>Pseudomonas</i>	-	-	-
12-27	2	<i>Moraxella</i>	-	-	-
12-28	2	<i>Moraxella</i>	-	-	-
12-29	2	coryneform	-	-	-
12-30	2	<i>A. caviae</i>	-	-	-
12-31	2	<i>P. mirabilis</i>	+	-	-
12-32	2	<i>A. caviae</i>	-	-	-
12-33	3	<i>S. putrefaciens</i>	-	-	-
12-34	3	<i>S. putrefaciens</i>	+	-	-
12-35	3	<i>Pseudomonas</i>	-	-	-
12-36	3	<i>Pseudomonas</i>	-	-	-
12-37	3	coryneform	-	-	-
12-38	3	<i>Moraxella</i>	-	-	-
12-39	3	<i>Lactobacillus</i>	-	-	+
12-40	3	coryneform	-	-	-
12-41	3	<i>Moraxella</i>	-	-	-

Table 6 - continued

Isolate Number	Days of Storage	Organism	Putrescine	Cadaverine	Indole
12-42	3	coryneform	-	-	-
12-43	4	<i>S. putrefaciens</i>	+	-	-
12-44	4	<i>Moraxella</i>	-	-	-
12-45	4	coryneform	-	-	-
12-46	4	<i>Moraxella</i>	-	-	-
12-47	4	coryneform	-	-	-
12-48	4	coryneform	-	-	-
12-49	4	<i>Moraxella</i>	-	-	-
12-50	4	<i>S. putrefaciens</i>	-	-	-
12-51	4	<i>S. putrefaciens</i>	-	-	-
12-52	4	<i>Alcaligenes</i>	-	-	-
12-53	4	coryneform	-	-	-
12-54	4	<i>Moraxella</i>	-	-	-
12-55	4	<i>Moraxella</i>	-	-	-
12-56	4	<i>Moraxella</i>	-	-	-

Table 7 - Production of putrescine, cadaverine, and indole by bacteria isolated from domestic shrimp decomposed at 24°C

Isolate Number	Hours of Storage	Organism	Putrescine	Cadaverine	Indole
24-1	0	<i>S. putrefaciens</i>	+	-	-
24-2	0	<i>F. odoratum</i>	-	-	-
24-3	0	coryneform	-	-	-
24-4	0	<i>Acinetobacter</i>	-	-	-
24-5	0	coryneform	-	-	-
24-6	0	<i>Pseudomonas fl/put</i>	-	-	-
24-7	0	coryneform	-	-	-
24-8	0	<i>Moraxella</i>	-	-	-
24-9	0	<i>Co. testosteroni</i>	-	+	-
24-10	0	<i>Moraxella</i>	-	-	-
24-11	0	coryneform	-	-	-
24-12	0	coryneform	-	-	-
24-13	12	coryneform	-	-	-
24-14	12	<i>Acinetobacter</i>	-	-	-
24-15	12	coryneform	-	-	-
24-16	12	<i>F. odoratum</i>	-	-	-
24-17	12	coryneform	-	-	-
24-18	12	coryneform	-	-	-
24-19	12	<i>Acinetobacter</i>	-	-	-
24-20	12	coryneform	-	-	-
24-21	12	coryneform	-	-	-
24-22	12	<i>Aeromonas</i>	-	-	+
24-23	12	coryneform	-	-	-
24-24	12	<i>Aeromonas</i>	+	-	+
24-25	24	<i>P. vulgaris</i>	-	-	+
24-26	24	<i>S. putrefaciens</i>	+	-	-
24-27	24	<i>F. odoratum</i>	-	-	-
24-28	24	<i>Bacillus</i>	-	-	-
24-29	24	coryneform	-	-	-
24-30	24	coryneform	-	-	-
24-31	24	<i>C. freundii</i>	-	-	-
24-32	24	coryneform	-	-	-
24-33	24	<i>Acinetobacter</i>	-	-	-
24-34	24	<i>Aeromonas</i>	+	-	-
24-35	24	<i>Acinetobacter</i>	-	-	-
24-36	24	coryneform	-	-	-
24-37	24	<i>F. odoratum</i>	-	-	-
24-38	24	<i>M. morganii</i>	+	-	+
24-39	24	coryneform	-	-	-

Table 7 - continued

Isolate Number	Hours of Storage	Organism	Putrescine	Cadaverine	Indole
24-40	36	<i>P. vulgaris</i>	-	-	+
24-41	36	<i>Bacillus</i>	-	-	-
24-42	36	<i>V. alginolyticus</i>	+	-	+
24-43	36	<i>P. vulgaris</i>	-	-	+
24-44	36	<i>M. morganii</i>	+	-	+
24-45	36	<i>F. odoratum</i>	-	-	+
24-46	36	coryneform	-	-	-
24-47	36	<i>F. odoratum</i>	-	-	-
24-48	36	Unidentified (dead)	-	-	-
24-49	36	<i>P. vulgaris</i>	-	-	+
24-50	36	<i>F. odoratum</i>	-	-	-
24-51	36	<i>P. vulgaris</i>	-	-	+
24-52	36	<i>M. morganii</i>	+	-	+
24-53	48	<i>P. vulgaris</i>	-	-	+
24-54	48	<i>Lactobacillus</i>	-	-	-
24-55	48	<i>M. morganii</i>	+	-	+
24-56	48	<i>F. odoratum</i>	-	-	-
24-57	48	<i>F. odoratum</i>	-	-	-
24-58	48	<i>F. odoratum</i>	-	-	-
24-59	48	<i>F. odoratum</i>	-	-	-
24-60	48	<i>Pasteurella haemolytica</i>	-	-	-
24-61	48	<i>M. morganii</i>	+	-	+
24-62	48	<i>P. vulgaris</i>	-	-	+
24-63	48	<i>Bacillus</i>	-	-	-
24-64	48	coryneform	-	-	-

Table 8 - Production of putrescine, cadaverine, and indole by bacteria isolated from domestic shrimp decomposed at 36°C

Isolate Number	Hours of Storage	Organism	Putrescine	Cadaverine	Indole
36-1	0	Unidentified (dead)	-	-	-
36-2	0	coryneform	-	-	-
36-3	0	<i>Pseudomonas</i>	-	-	-
36-4	0	coryneform	-	-	-
36-5	0	coryneform	-	-	-
36-6	0	coryneform	-	-	-
36-7	0	coryneform	-	-	-
36-8	0	<i>Acinetobacter</i>	-	-	-
36-9	0	<i>Co. testosteroni</i>	-	+	-
36-10	0	coryneform	-	-	-
36-11	0	coryneform	-	-	-
36-12	0	<i>St. maltophilia</i>	-	-	-
36-13	0	<i>S. putrefaciens</i>	-	-	-
36-14	0	coryneform	-	-	-
36-15	0	<i>V. parahaemolyticus</i>	+	-	+
36-16	0	coryneform	-	-	-
36-17	0	<i>P. vulgaris</i>	-	-	+
36-18	6	<i>Bacillus</i>	-	-	-
36-19	6	<i>Ph. damsela</i>	-	-	-
36-20	6	<i>Pseudomonas</i>	-	-	-
36-21	6	<i>Aeromonas</i>	-	-	+
36-22	6	<i>V. alginolyticus</i>	-	-	-
36-23	6	<i>Lactobacillus</i>	-	-	-
36-24	6	CDC Gr. EF-4	-	-	-
36-25	6	<i>C. braakii</i>	+	-	-
36-26	6	<i>Bacillus</i>	-	-	+
36-27	6	<i>Lactobacillus</i>	-	-	-
36-28	6	coryneform	-	-	-
36-29	6	Unidentified	-	-	-
36-30	12	<i>M. organii</i>	+	-	-
36-31	12	<i>Aeromonas</i>	-	-	+
36-32	12	<i>Ph. damsela</i>	-	+	-
36-33	12	<i>Pa. haemolytica</i>	-	-	-
36-34	12	<i>S. putrefaciens</i>	-	-	-
36-35	12	<i>Staphylococcus</i>	-	-	-
36-36	12	<i>V. parahaemolyticus</i>	+	+	+
36-37	12	<i>M. organii</i>	+	-	+
36-38	12	<i>M. organii</i>	+	-	+
36-39	12	<i>Aeromonas</i>	+	-	+

Table 8 - continued

Isolate Number	Hours of Storage	Organism	Putrescine	Cadaverine	Indole
36-40	12	<i>P. vulgaris</i>	-	-	+
36-41	18	<i>P. vulgaris</i>	-	-	+
36-42	18	<i>S. putrefaciens</i>	+	-	-
36-43	18	<i>F. odoratum</i>	-	-	-
36-44	18	<i>V. alginolyticus</i>	+	+	+
36-45	18	coryneform	-	-	-
36-46	18	<i>M. morgani</i>	+	-	+
36-47	18	<i>P. mirabilis</i>	+	-	-
36-48	18	<i>F. odoratum</i>	-	-	-
36-49	24	<i>P. vulgaris</i>	-	-	+
36-50	24	coryneform	-	-	-
36-51	24	<i>Bacillus</i>	-	-	-
36-52	24	<i>M. morgani</i>	+	-	+
36-53	24	<i>V. alginolyticus</i>	+	+	+
36-54	24	<i>Eikenella corrodans</i>	-	-	-
36-55	24	<i>Alcaligenes</i>	-	-	-
36-56	24	<i>P. stutzeri</i>	-	-	-
36-57	24	<i>S. putrefaciens</i>	+	-	-
36-58	24	<i>Lactobacillus</i>	-	-	-
36-59	24	<i>M. morgani</i>	+	-	+
36-60	24	Unidentified (dead)	-	-	-

Chemical and Sensory Analyses

Mean putrescine, cadaverine, and indole concentrations and degree of sensory decomposition for domestic Penaeid shrimp (*L. setiferus*) decomposed at 0 and 12°C are shown in Figures 1 and 2, respectively. Mean putrescine, cadaverine, and indole concentrations and degree of sensory decomposition for domestic Penaeid shrimp (*L. brasiliensis*) decomposed at 24 and 36°C are shown in Figures 3 and 4, respectively. These figures show that decomposition progressed more rapidly with time at progressively higher temperatures. The shrimp were considered to be decomposed by sensory evaluation on day 12 at 0°C, on day 2 at 12°C, at hour 12 at 24°C, and at hour 6 at 36°C.

Chemical analyses indicated that putrescine, cadaverine, and indole levels increased with time at each storage temperature. The putrescine and cadaverine data generally agree with previous research by Shakila et al. (1995) which showed overall increases in putrescine and cadaverine concentrations in Indian shrimp (*Parapenaeopsis stylifera*) stored at 0 and 5°C, however, the putrescine and cadaverine concentrations increased to higher levels in our shrimp. Additionally, the cadaverine concentrations detected in shrimp decomposed at 30°C showed an initial increase followed by a gradual decrease over the 24-hour storage period (Shakila et al., 1995). The indole data are also in agreement with previous studies conducted on shrimp decomposed at various temperatures (Matches, 1982; Chang et al., 1983; and Shamshad, et al., 1990). These researchers showed minimal increases in indole concentrations at temperatures less than 10°C and more rapid indole concentrations increases at higher temperatures.

Mean putrescine levels increased from 1.4 to 77.8 ppm, cadaverine levels increased from 0 to 2.2 ppm, and indole levels increased from 0 to 21.2 µg/100g for domestic shrimp (*L. setiferus*) decomposed at 0°C for 16 days. Mean putrescine levels increased from 1.4 to 613.5 ppm, cadaverine levels increased from 0 to 6.0 ppm, and indole levels increased from 0 to 114.1 µg/100g for domestic shrimp (*L. setiferus*) decomposed at 12°C for 4 days. Mean putrescine levels increased from 9.1 to 2,575.3 ppm, cadaverine levels increased from 0 to 297.0 ppm, and indole levels increased from 0 to 2,640.0 µg/100g for domestic shrimp (*L. brasiliensis*) decomposed at 24°C for 48 hours. Mean putrescine levels increased from 9.1 to 1,765.8 ppm, cadaverine levels increased from 0 to 482.8 ppm, and indole levels increased from 0 to 2,577.6 µg/100g for domestic shrimp (*L. brasiliensis*) decomposed at 36°C for 24 hours.

The general increase of putrescine, cadaverine, and indole concentrations combined with sensory evaluations at each temperature of storage suggests the potential for putrescine, cadaverine, and indole to be effective chemical indicators of decomposition in Penaeid shrimp.

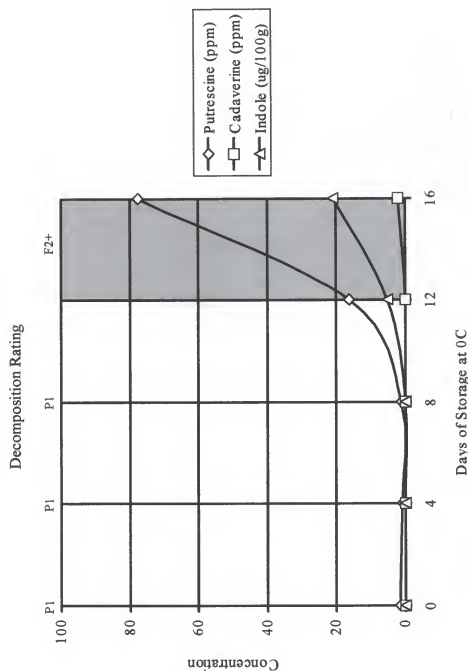


Figure 1 – Mean putrescine, cadaverine, and indole concentrations and degree of sensory decomposition in domestic Penaeid shrimp (*L. setiferus*) decomposed at 0°C. (Increased shading denotes progressive sensory decomposition; P1 = Pass Class 1 and F2+ = Fail Class 2 and greater.)

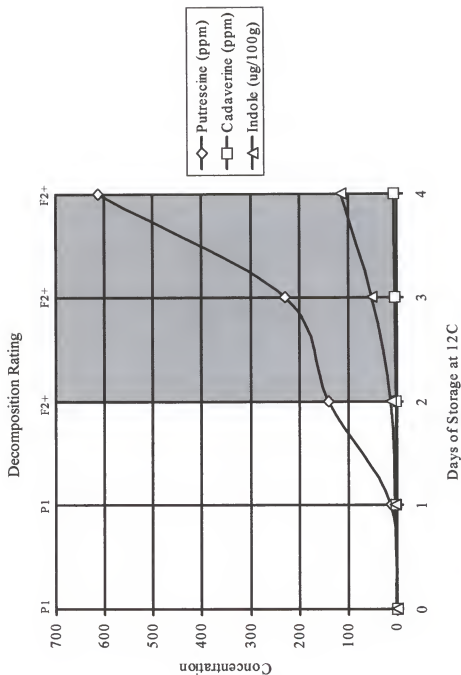


Figure 2 – Mean putrescine, cadaverine, and indole concentrations and degree of sensory decomposition in domestic Penaeid shrimp (*L. setiferus*) decomposed at 12°C. (Increased shading denotes progressive sensory decomposition; P1 = Pass Class 1 and F2+ = Fail Class 2 and greater.)

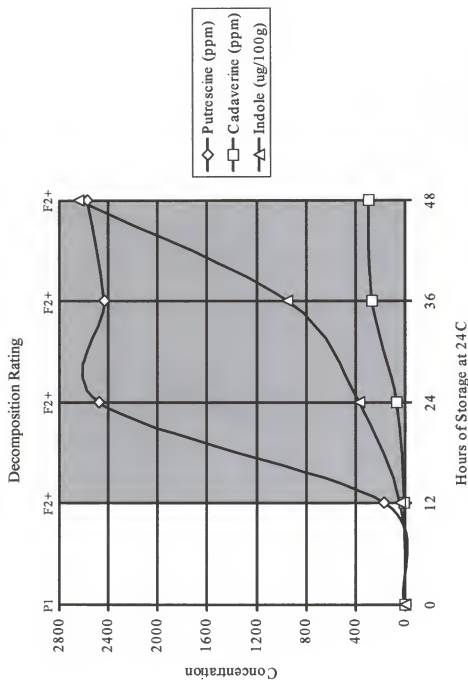


Figure 3 – Mean putrescine, cadaverine, and indole concentrations and degree of sensory decomposition in domestic Penaeid shrimp (*L. brasiliensis*) decomposed at 24°C. (Increased shading denotes progressive sensory decomposition; P1 = Pass Class 1 and F2+ = Fail Class 2 and greater.)

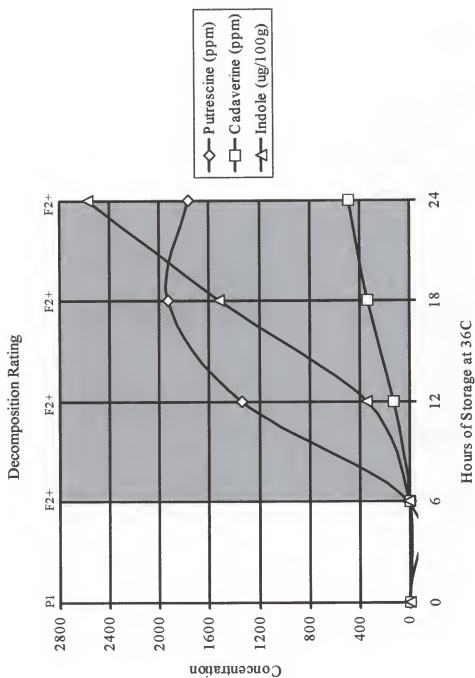


Figure 4 – Mean putrescine, cadaverine, and indole concentrations and degree of sensory decomposition in domestic Penaeid shrimp (*L. brasiliensis*) decomposed at 36°C. (Increased shading denotes progressive decomposition; P1 = Pass Class 1 and F2+ = Fail Class 2 and greater.)

Nicaraguan Shrimp Decomposition

Microbiological Analyses

Mean aerobic plate counts (APC- $\log_{10}/g$) and microbial type characterizations for aquacultured Nicaraguan shrimp (*Litopenaeus vannamei*) decomposed at 0°C for 20 days and 12°C for 4 days are shown in Table 9. The APCs ($\log_{10}/g$) of the shrimp increased as expected over the 16 and 4-day storage periods. Mean APCs ($\log_{10}/g$) increased from 4.8 to 9.0 and 4.7 to 8.6 for the 0 and 12°C decomposed shrimp, respectively. The results for decomposition at 0°C generally agree with previous research on aquacultured shrimp. Christopher et al. (1978) reported that fresh pond-raised *P. stylirostris*, *P. vannamei*, and *P. setiferus* had APC's ranging from 1.5×10^3 to 1.3×10^4 . Additionally, pond-raised shrimp (*P. aztecus*, *P. occidentalis*, *P. setiferus*, and *P. vannamei*) were reported to have initial APCs ranging from 10^4 to 10^5 with counts increasing to as high as 10^7 after 28 days on ice (Vanderant et al., 1973). No studies on aquacultured shrimp decomposed at 12°C have been published. However, Chang et al. (1983) showed APCs increasing from 10^5 to 10^8 for wild-caught *P. setiferus* and *P. duorarum* stored for 72 hours at 12°C.

The microbial type characterization of aquacultured Nicaraguan shrimp stored at 0°C showed a microflora initially dominated by *S. putrefaciens* and coryneform bacteria with lower levels of *Pasteurella haemolytica*, *V. alginolyticus*, CDC Group EF-4, *Lactobacillus*, and *V. vulnificus* detected. By day 8 *P. haemolytica* and *Moraxella* dominated the microflora with lower levels of *Lactobacillus*, coryneform bacteria, unidentified bacteria, and *Acinetobacter* isolated. On day 16 *P. haemolytica* and *P. fluorescens/putida* were prevalent and lower levels of *Lactobacillus* and coryneform bacteria were detected. By day 20 the microflora consisted of *P. haemolytica* (43%), *P.*

fluorescens/putida (35%), *Lactobacillus* (20%), *P. multocida* (1%), and *S. putrefaciens* (1%). These results differ somewhat from a previous study on microbial type changes in pond-raised shrimp during storage at 0°C (Vanderzant et al., 1973) in that the initial microflora in their study consisted of *Aeromonas*, *Pseudomonas*, and *Vibrio* which changed to a microflora dominated by coryneform bacteria by day 21. These differences may have resulted from the fact that their shrimp were raised during 1972 in Texas (USA) and our shrimp were raised during 1998-99 in Nicaragua which could account for the different microbial type characterizations determined initially and during storage for these two studies.

The microbial type characterization of aquacultured shrimp stored at 12°C showed a microflora initially dominated by *S. putrefaciens* with lower levels of coryneform bacteria, *Chryseobacterium/Flavobacterium indologenes*, *P. haemolytica*, *Acinetobacter*, and *P. fluorescens/putida* detected. On day 2 the microflora consisted of *P. haemolytica* (58%), coryneform bacteria (25%), *Lactobacillus* (11%), and *Aeromonas* (6%). By day 4 the microflora was dominated by *P. haemolytica* and coryneform bacteria with lower levels of *S. putrefaciens*, *Proteus vulgaris*, and CDC Group EF-4 isolated. Our research differs from previous studies on the microflora of shrimp decomposed at temperatures at or near 12°C but these studies were conducted on wild-caught product from different parts of the world (Matches, 1983; Shamshad et al., 1990). Matches (1982) research was conducted on *Pandalus jordani* caught off the coast of Washington (USA) and Shamshad et al. (1990) worked on *Penaeus merguensis* from Pakistan.

Mean aerobic plate counts (APC- $\log_{10}/g$) and microbial type characterizations for aquacultured Nicaraguan shrimp (*Litopenaeus vannamei*) decomposed at 24°C for 24 hours and 36°C for 12 hours are shown in Table 10. The APCs ($\log_{10}/g$) of the shrimp increased as expected over the 24 and 12-hour storage periods. Mean APCs ($\log_{10}/g$) increased from 4.5 to 8.5 and 4.7 to 8.0 for the 24 and 36°C decomposed shrimp, respectively. Research involving aquacultured shrimp decomposed at temperatures at or near 24 and 36°C has not been published, however, Chang et al. (1983) reported APCs increasing from 10^5 to 10^9 for wild-caught *P. setiferus* and *P. duorarum* stored for 24 hours at 24°C and Shamshad et al. (1990) reported counts increasing from 10^5 to 10^9 for *Penaeus merguensis* decomposed at 35°C for 24 hours.

The microbial type characterization of aquacultured shrimp stored at 24°C showed a microflora initially dominated by coryneform bacteria and *S. putrefaciens* with lower levels of *Chry./Fl. indologenes*, *Bacillus*, and unidentified bacteria detected. At hour 12 *P. haemolytica* was the prevalent microorganism isolated with lower levels of *V. vulnificus*, *V. parahaemolyticus*, *S. putrefaciens*, *Chry./Fl. indologenes*, *V. alginolyticus*, and *V. fluvialis* detected. By hour 24 the microflora was dominated by *V. parahaemolyticus* with lower levels of *P. haemolytica*, *P. damsela*, *S. putrefaciens*, *V. alginolyticus*, coryneform bacteria, *Lactobacillus*, and unidentified bacteria detected.

The microbial type characterization of aquacultured shrimp stored at 36°C showed a microflora initially dominated by *S. putrefaciens* with lower levels of *Bacillus*, *P. haemolytica*, coryneform bacteria, *Pseudomonas* spp., and *V. alginolyticus* detected. At hour 6 *V. alginolyticus*, *V. vulnificus*, and *S. putrefaciens* comprised the majority of the microorganisms isolated with lower levels of *Bacillus*, *P. haemolytica*, *V. fluvialis*,

and *V. parahaemolyticus* detected. By hour 12 *V. parahaemolyticus* was the prevalent organism isolated with lower levels of *P. damsela*, *S. putrefaciens*, *P. haemolytica*, *V. vulnificus*, *V. alginolyticus*, *Bacillus*, and coryneform bacteria detected. Although no research associated with the bacterial population dynamics on aquacultured shrimp decomposed at or near 24 and 36°C has been published, Shamshad et al. (1990) showed similar results to ours in that the microflora of wild-caught *P. merguensis* stored at 25 and 35°C was dominated by *Vibrio* spp. by the end of the storage period.

The abilities of bacteria isolated from aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 0, 12, 24, and 36°C to produce putrescine from ornithine, cadaverine from lysine, and indole from tryptophan are listed in Tables 11-14. A total of 116 bacteria were isolated and identified. Bacteria capable of producing putrescine from ornithine were *S. putrefaciens* (2) and *P. multocida* (1) isolated from shrimp decomposed at 0°C, *S. putrefaciens* (1) isolated from shrimp decomposed at 12°C, *S. putrefaciens* (4), *V. parahaemolyticus* (4), *V. alginolyticus* (2), and *V. fluvialis* (1) isolated from shrimp decomposed at 24°C, and *S. putrefaciens* (3), *V. parahaemolyticus* (3), and *V. alginolyticus* (2) isolated from shrimp decomposed at 36°C.

Microorganisms capable of producing cadaverine from lysine were coryneform bacteria (1) isolated from shrimp decomposed at 0°C, *Aeromonas sobria* (1) and CDC Group EF-4 (1) isolated from shrimp decomposed at 12°C, *V. parahaemolyticus* (4), *V. alginolyticus* (2), *V. fluvialis* (1), and *P. damsela* (1) isolated from shrimp decomposed at 24°C, and *V. alginolyticus* (5) and *V. parahaemolyticus* (5) isolated from shrimp decomposed at 36°C.

Bacteria capable of producing indole from tryptophan were *V. alginolyticus* (1), *V. vulnificus* (1), and unidentified bacteria (1) isolated from shrimp decomposed at 0°C, *Chry./Fl. indologenes* (1), *A. sobria* (1), and *P. vulgaris* (1) isolated from shrimp decomposed at 12°C, *V. parahaemolyticus* (4), *V. alginolyticus* (2), *Chry./Fl. indologenes* (2), unidentified bacteria (2), *V. fluvialis* (1), and *V. vulnificus* (1) isolated from shrimp decomposed at 24°C, and *V. alginolyticus* (8), *V. parahaemolyticus* (6), *V. vulnificus* (2), and *V. fluvialis* (1) isolated from shrimp decomposed at 36°C.

The data listed in Tables 11-14 indicate that *S. putrefaciens* was the major microorganism responsible for putrescine production in the shrimp decomposed at 0 and 12°C while *S. putrefaciens* and *Vibrio* spp. were jointly responsible for putrescine production in shrimp decomposed at 24 and 36°C. This data generally agrees with previous research conducted on shrimp decomposed at similar temperatures in that psychrotrophic microorganisms (i.e., *Pseudomonas* and *Shewanella*) tend to dominate the microflora of shrimp decomposed at lower temperatures while mesophilic microorganisms (i.e., *Vibrio* spp.) become more prevalent in shrimp decomposed at higher temperatures (Matches, 1982; Vanderzant et al., 1973; Shamshad et al., 1990).

The data listed in Tables 11-14 also indicate that no single organism or group of organisms isolated was dominantly responsible for potential cadaverine or indole production in shrimp decomposed at 0 and 12°C while *Vibrio* spp. (mainly *V. alginolyticus* and *V. parahaemolyticus*) were clearly dominant cadaverine and indole producers on shrimp decomposed at 24 and 36°C. Again we see that the mesophilic microorganisms dominated the microflora at higher decomposition temperatures.

The above results showing putrescine production by *S. putrefaciens* isolated from shrimp decomposed at 0 and 12°C and putrescine production by *S. putrefaciens* and *Vibrio* spp. isolated from shrimp decomposed at 24 and 36°C indicate the microbiological potential for putrescine to be an effective chemical indicator of decomposition for shrimp decomposed over a wide temperature range. Additionally, the above results showing a lack of dominant cadaverine or indole producing isolates in shrimp decomposed at 0 and 12°C indicate that microbiological limitations may hinder the effectiveness of cadaverine and/or indole as chemical indicators in shrimp decomposed at lower temperatures. However, the prevalence of cadaverine and indole producing *Vibrio* spp. isolated from shrimp decomposed at 24 and 36°C indicate the microbiological potential for cadaverine and indole to be effective chemical indicators of shrimp decomposed at higher temperatures.

Table 9 - Decomposition ratings, mean putrescine, cadaverine, indole levels, aerobic plate counts (log₁₀/g), and percent characterization of microbial types for aquacultured Nicaraguan Penaeid shrimp (*L. vannamei*) stored at 0 and 12°C^{a,b}

	0°C				12°C	
	Day 0 P1	Day 8 P1	Day 16 F2	Day 20 F2	Day 0 P1	Day 2 LQP
Decomposition Rating						
Putrescine ppm	0.3 ±0.3	0.4 ±0.1	7.0 ±5.2	19.6 ±7.1	0.1 ±0.1	0.1 ±0.2
Cadaverine ppm	0.4 ±0.3	1.0 ±0.3	0.6 ±0.1	1.0 ±0.3	0.7 ±0.4	0.8 ±0.4
Indole µg/100g	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0
APC(log ₁₀)	4.8	5.8	8.8	9.0	4.7	7.1
Organism	Percent Distribution of Microbial Types					
<i>Acinetobacter</i>		2			2	
<i>Aeromonas</i>						6 (C,I)
<i>Bacillus</i>						
CDC Gr. EF-4	2					2 (C)
<i>Chry./Fl. indologenes</i>					5 (I)	
<i>coryneforms</i>	25	4	11 (C)		11	25
<i>Flavobacterium</i>						
<i>Lactobacillus</i>	2	15	14	20		11
<i>Moraxella</i>		30				
<i>Pa. haemolytica</i>	11	45	51	43	5	58
<i>Pa. multocida</i>				1 (P)		
<i>P. vulgaris</i>						3 (I)
<i>Pseudomonas</i>						
<i>Pseudomonas fl/put</i>			24	35	2	
<i>S. purefaciens</i>	54 (P)			1 (P)	75 (P)	6 (P)

Table 9 - continued

Organism	0°C				12°C		
	Day 0	Day 8	Day 16	Day 20	Day 0	Day 2	Day 4
	Percent Distribution of Microbial Types						
<i>V. alginolyticus</i>	4 (I)						
<i>V. parahaemolyticus</i>							
<i>V. vulnificus</i>	2 (I)						
Unidentified		4 (I)					

^aThe letters in parentheses indicate microbial isolates capable of producing putrescine (P), cadaverine (C), and indole (I).

^bNumbers following the ± sign are the standard deviations of the means of putrescine (ppm), cadaverine (ppm), and indole (µg/100g) listed in each cell.

Table 10 - Decomposition ratings, mean putrescine, cadaverine, indole levels, aerobic plate counts ($\log_{10}/g$), and percent characterization of microbial types for aquacultured Nicaraguan Penaeid shrimp (*L. vannamei*) stored at 24 and 36°C^{a,b}

Decomposition Rating	24°C			36°C		
	Hour 0 P1	Hour 12 F2	Hour 24 F3	Hour 0 P1	Hour 6 F2	Hour 12 F3
Putrescine ppm	0.4 ±0.2	11.8 ±1.8	336.6 ±27.3	0.1 ±0.1	10.3 ±8.2	347.0 ±29.5
Cadaverine ppm	0.6 ±0.1	16.1 ±2.1	110.9 ±8.7	0.4 ±0.5	15.0 ±6.3	113.3 ±11.2
Indole µg/100g	0 ±0	3.3 ±4.3	60.6 ±10.6	0 ±0	3.3 ±0.1	54.7 ±14.2
APC ^(log)	4.5	6.4	8.5	4.7	6.5	8.0
Organism	Percent Distribution of Microbial Types					
<i>Acinetobacter</i>						
<i>Aeromonas</i>						
<i>Bacillus</i>	3			18	4	1
CDC Gr. EF-4						
<i>Chry./Fl. indologenes</i>	14 (1)	3 (1)				
<i>Coryneforms</i>	45		3	9		1
<i>F. odoratum</i>						
<i>Lactobacillus</i>			3			
<i>Moraxella</i>						
<i>Pa. haemolytica</i>		66	17	11	4	6
<i>Pa. multocida</i>			5 (C)			15
<i>Ph. damsela</i>						
<i>P. vulgaris</i>						
<i>Pseudomonas</i>				5		
<i>Pseudomonas fl/put</i>						

Table 10 - continued

Organism	24°C		36°C	
	Hour 0	Hour 12	Hour 0	Hour 12
	Percent Distribution of Microbial Types			
<i>S. putrefaciens</i>	35 (P)	7 (P)	55 (P)	7 (P)
<i>V. alginolyticus</i>		3 (P,C,I)	21 (P)	3 (P,C,I)
<i>V. fluvialis</i>		2 (P,C,I)	42 (P,C,I)	4 (I)
<i>V. parahaemolyticus</i>		9 (P,C,I)	4 (P,C,I)	61 (P,C,I)
<i>V. vulnificus</i>		10 (I)	21 (I)	6 (I)
Unidentified	3 (I)			

^aThe letters in parentheses indicate microbial isolates capable of producing putrescine (P), cadaverine (C), and indole (I).

^bNumbers following the ± sign are the standard deviations of the means of putrescine (ppm), cadaverine (ppm), and indole (µg/100g) listed in each cell.

Table 11 - Production of putrescine, cadaverine, and indole by bacteria isolated from aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 0°C

Isolate Number	Days of Storage	Organism	Putrescine	Cadaverine	Indole
N0-1	0	<i>S. putrefaciens</i>	+	-	-
N0-2	0	<i>Pa. haemolytica</i>	-	-	-
N0-3	0	<i>V. vulnificus</i>	-	-	+
N0-4	0	coryneform	-	-	-
N0-5	0	<i>Lactobacillus</i>	-	-	-
N0-6	0	CDC Group EF-4	-	-	-
N0-7	0	<i>V. alginolyticus</i>	-	-	+
N0-8	0	coryneform	-	-	-
N0-9	0	<i>Pa. multocida</i>	-	-	-
N0-10	8	<i>Pa. haemolytica</i>	-	-	-
N0-11	8	coryneform	-	-	-
N0-12	8	<i>Lactobacillus</i>	-	-	-
N0-13	8	<i>Moraxella</i>	-	-	-
N0-14	8	Unidentified (dead)	-	-	+
N0-15	8	<i>Acinetobacter</i>	-	-	-
N0-16	8	Unidentified (dead)	-	-	-
N0-17	16	<i>Pa. haemolytica</i>	-	-	-
N0-18	16	<i>P. fluorescens</i>	-	-	-
N0-19	16	coryneform	-	+	-
N0-20	16	coryneform	-	-	-
N0-21	16	<i>Lactobacillus</i>	-	-	-
N0-22	16	<i>Pa. haemolytica</i>	-	-	-
N0-23	16	<i>Lactobacillus</i>	-	-	-
N0-24	20	<i>Pa. haemolytica</i>	-	-	-
N0-25	20	<i>Pseudomonas fl/put</i>	-	-	-
N0-26	20	<i>S. putrefaciens</i>	+	-	-
N0-27	20	<i>Pa. multocida</i>	+	-	-
N0-28	20	<i>Lactobacillus</i>	-	-	-
N0-29	20	<i>Lactobacillus</i>	-	-	-

Table 12 - Production of putrescine, cadaverine, and indole by bacteria isolated from aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 12°C

Isolate Number	Days of Storage	Organism	Putrescine	Cadaverine	Indole
N12-1	0	<i>S. putrefaciens</i>	+	-	-
N12-2	0	<i>Pa. haemolytica</i>	-	-	-
N12-3	0	coryneform	-	-	-
N12-4	0	<i>Ch/Fl. indologenes</i>	-	-	+
N12-5	0	coryneform	-	-	-
N12-6	0	coryneform	-	-	-
N12-7	0	coryneform	-	-	-
N12-8	0	<i>Acinetobacter</i>	-	-	-
N12-9	0	<i>Pseudomonas fl/put</i>	-	-	-
N12-10	2	<i>Pa. haemolytica</i>	-	-	-
N12-11	2	coryneform	-	-	-
N12-12	2	coryneform	-	-	-
N12-13	2	<i>Lactobacillus</i>	-	-	-
N12-14	2	<i>Lactobacillus</i>	-	-	-
N12-15	2	<i>A. sobria</i>	-	+	+
N12-16	4	<i>S. putrefaciens</i>	+	-	-
N12-17	4	coryneform	-	-	-
N12-18	4	<i>Pa. haemolytica</i>	-	-	-
N12-19	4	<i>P. vulgaris</i>	-	-	+
N12-20	4	coryneform	-	-	-
N12-21	4	<i>Pa. haemolytica</i>	-	-	-
N12-22	4	CDC Group EF-4	-	+	-
N12-23	4	<i>Pa. haemolytica</i>	-	-	-
N12-24	4	<i>Pa. haemolytica</i>	-	-	-

Table 13 - Production of putrescine, cadaverine, and indole by bacteria isolated from aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 24°C

Isolate Number	Hours of Storage	Organism	Putrescine	Cadaverine	Indole
N24-1	0	<i>S. putrefaciens</i>	+	-	-
N24-2	0	Unidentified (dead)	-	-	+
N24-3	0	coryneform	-	-	-
N24-4	0	<i>Ch/Fl. indologenes</i>	-	-	+
N24-5	0	coryneform	-	-	-
N24-6	0	coryneform	-	-	-
N24-7	0	coryneform	-	-	-
N24-8	0	coryneform	-	-	-
N24-9	0	<i>Bacillus</i>	-	-	-
N24-10	12	<i>V. vulnificus</i>	-	-	+
N24-11	12	<i>S. putrefaciens</i>	+	-	-
N24-12	12	<i>Pa. haemolytica</i>	-	-	-
N24-13	12	<i>Ch/Fl. indologenes</i>	-	-	+
N24-14	12	<i>S. putrefaciens</i>	+	-	-
N24-15	12	<i>V. fluvialis</i>	+	+	+
N24-16	12	<i>V. parahaemolyticus</i>	+	+	+
N24-17	12	<i>Pa. haemolytica</i>	-	-	-
N24-18	12	<i>V. alginolyticus</i>	-	-	+
N24-19	12	<i>V. alginolyticus</i>	+	+	-
N24-20	24	<i>V. parahaemolyticus</i>	+	+	+
N24-21	24	<i>Ph. damsela</i>	-	+	-
N24-22	24	Unidentified (dead)	-	-	+
N24-23	24	<i>V. parahaemolyticus</i>	+	+	+
N24-24	24	<i>Pa. haemolytica</i>	-	-	-
N24-25	24	<i>Pa. haemolytica</i>	-	-	-
N24-26	24	<i>S. putrefaciens</i>	+	-	-
N24-27	24	<i>V. alginolyticus</i>	+	+	+
N24-28	24	<i>Lactobacillus</i>	-	-	-
N24-29	24	<i>V. parahaemolyticus</i>	+	+	+
N24-30	24	coryneform	-	-	-

Table 14 - Production of putrescine, cadaverine, and indole by bacteria isolated from aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 36°C

Isolate Number	Hours of Storage	Organism	Putrescine	Cadaverine	Indole
N36-1	0	<i>S. putrefaciens</i>	+	-	-
N36-2	0	<i>Bacillus</i>	-	-	-
N36-3	0	<i>V. alginolyticus</i>	-	-	+
N36-4	0	<i>Pa. haemolytica</i>	-	-	-
N36-5	0	<i>Pa. haemolytica</i>	-	-	-
N36-6	0	<i>Pseudomonas</i>	-	-	-
N36-7	0	coryneform	-	-	-
N36-8	0	coryneform	-	-	-
N36-9	6	<i>V. alginolyticus</i>	-	+	+
N36-10	6	<i>V. alginolyticus</i>	-	-	+
N36-11	6	<i>V. vulnificus</i>	-	-	+
N36-12	6	<i>S. putrefaciens</i>	+	-	-
N36-13	6	<i>Pa. haemolytica</i>	-	-	-
N36-14	6	<i>Bacillus</i>	-	-	-
N36-15	6	<i>V. parahaemolyticus</i>	+	+	+
N36-16	6	<i>V. alginolyticus</i>	-	+	+
N36-17	6	<i>V. alginolyticus</i>	+	+	+
N36-18	6	<i>V. fluvialis</i>	-	-	+
N36-19	6	<i>V. alginolyticus</i>	-	+	+
N36-20	12	<i>V. parahaemolyticus</i>	+	+	+
N36-21	12	<i>V. parahaemolyticus</i>	-	+	+
N36-22	12	<i>S. putrefaciens</i>	+	-	-
N36-23	12	<i>V. vulnificus</i>	-	-	+
N36-24	12	coryneform	-	-	-
N36-25	12	<i>Pa. haemolytica</i>	-	-	-
N36-26	12	<i>Ph. damsela</i>	-	-	-
N36-27	12	<i>S. putrefaciens</i>	+	-	-
N36-28	12	<i>V. parahaemolyticus</i>	-	+	+
N36-29	12	<i>Bacillus</i>	-	-	-
N36-30	12	<i>V. parahaemolyticus</i>	-	-	+
N36-31	12	<i>V. parahaemolyticus</i>	+	+	+
N36-32	12	<i>V. alginolyticus</i>	-	+	+
N36-33	12	<i>V. alginolyticus</i>	+	+	+

Chemical and Sensory Analyses

Graphs of mean putrescine, cadaverine, and indole concentrations in aquacultured Nicaraguan Penaeid shrimp (*L. vannamei*) decomposed at 0, 12, 24, and 36°C with the y-axis reduced to focus on chemical changes occurring during the transition from acceptable to unacceptable product as judged by sensory evaluation are shown in Figures 5-8, respectively. Since some indicator trendlines extend beyond the maximum value of the y-axis it should be noted that in each case the chemical indicator represented by the trendline continued to increase throughout the storage period. In general, the data show that decomposition progressed more rapidly with time at progressively higher temperatures. And more specifically, the shrimp were considered to be decomposed by sensory evaluation on day 14 at 0°C, on day 2.5 at 12°C, at hour 10 at 24°C, and at hour 6 at 36°C.

Chemical analyses indicated that putrescine, cadaverine, and indole levels increased with time at each storage temperature with the exception that no indole was detected in the shrimp decomposed at 0°C. The putrescine and cadaverine data generally agree with previous research by Shakila et al. (1995) which showed overall increases in putrescine and cadaverine concentrations in Indian shrimp (*Parapenaeopsis stylifera*) stored at 0 and 5°C however, the cadaverine concentrations detected in shrimp decomposed at 30°C showed an initial increase followed by a gradual decrease over the 24-hour storage period. The indole data are also in agreement with previous studies conducted on shrimp decomposed at various temperatures (Matches, 1982; Chang et al., 1983; and Shamshad, et al., 1990). These researchers showed minimal increases in indole concentrations at temperatures less than 10°C and more rapid indole concentration

increases at higher temperatures. All of these researchers detected indole in shrimp decomposed at 0°C which differs from the results of this study. The absence of indole in our shrimp decomposed at 0°C can be explained by the fact that the shrimp used in this study were taken from the pond and placed directly in ice slush while still alive. Thus, indole-producing microorganisms, which generally grow at higher temperatures, were never allowed the opportunity to grow. Placing live shrimp in ice slush for expiration is a practice unique to the aquacultured shrimp industry. Trawled shrimp generally remain in the harvest net for a period of time, are dumped on the boat deck and separated from the by-catch, the heads are removed, and then they are placed on ice or are brine frozen for storage.

Mean putrescine levels increased from 0.3 to 19.6 ppm, cadaverine levels increased from 0.4 to 3.3 ppm, and indole was not detected in aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 0°C for 20 days. Mean putrescine levels increased from 0.1 to 193.9 ppm, cadaverine levels increased from 0.7 to 46.0 ppm, and indole levels increased from 0 to 4.1 µg/100g for aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 12°C for 4.5 days. Mean putrescine levels increased from 0.4 to 336.6 ppm, cadaverine levels increased from 0.6 to 110.9 ppm, and indole levels increased from 0 to 60.6 µg/100g for aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 24°C for 24 hours. Finally, mean putrescine levels increased from 0.1 to 347.0 ppm, cadaverine levels increased from 0.4 to 113.3 ppm, and indole levels increased from 0 to 54.7 µg/100g for aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 36°C for 12 hours.

Sensory evaluations of aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 0°C for 20 days indicated P1 quality product until day 12 when the shrimp were classified as BLP. On day 14 the shrimp had progressed to the BLF category and by day 16 the product was classified as F2 where it remained through day 20. Aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 12°C held a P1 quality rating until day 2 when they were classified as LQP. By day 2.5 the product had progressed to the F2 classification where it remained until day 4 when it was assigned an F2 Adv rating. The shrimp was also classified as F2 Adv on day 4.5. Aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 24°C was classified as P1 until hour 8 when it fell into the LQP category. At hour 10 the product was degraded to the BLF level and by hours 12 and 14 had received F2 classifications. An F2 Adv rating was assigned to the shrimp by hour 16 and at hours 20 and 24 the product was classified as F3. Aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 36°C held a P1 rating until hour 4 when it was classified in the BLP category. At hour 6 the product was classified as F2 quality followed by F3 ratings at hours 8, 10, and 12.

The most significant trend displayed on graphs 5-8 was the rapid increase in the putrescine concentration above the 3-ppm level at or near the sensory failure point for all four decomposition temperatures. This result was especially evident for decomposition at 12, 24, and 36°C, where the transition from acceptable to unacceptable product was directly linked to an increase in putrescine above the 3-ppm level. This shows that putrescine concentration at the 3-ppm level was an effective chemical indicator of decomposition for shrimp decomposed at 12, 24, and 36°C.

At the 0°C decomposition temperature the putrescine concentration increased above the 3-ppm level one sample increment (2 days) after the product was considered to be unacceptable by sensory evaluation. However, the product was rated as BLF on day 14 where the mean putrescine concentration was 0.9 ppm and rated F2 on day 16 where the putrescine concentration was 7.0 ppm. This shows that putrescine concentration at the 3-ppm level was still an effective chemical indicator of decomposition for shrimp decomposed at 0°C but the level increased slightly after sensory decomposition was detected.

The same result was also evident for cadaverine concentrations in aquacultured Nicaraguan shrimp decomposed at 12, 24, and 36°C. At each of these decomposition temperatures cadaverine concentrations increased slightly more rapidly than the putrescine concentrations and were clearly above the 3-ppm level at the point where the product reached sensory unacceptability. This demonstrates that a cadaverine concentration above the 3-ppm level was also a very effective chemical indicator of decomposition for shrimp decomposed at or above 12°C.

At the 0°C decomposition temperature the mean cadaverine concentration was only detected above the 3-ppm level (3.3 ppm) on day 18 of storage. Subsequently the mean cadaverine concentration decreased to below the 3-ppm level (1.0 ppm) in the shrimp on day 20. This demonstrates an inconsistent trend and restricts the effectiveness of cadaverine concentration as a chemical indicator of decomposition in shrimp decomposed at 0°C. Very limited data concerning sensory ratings, putrescine, and cadaverine levels in shrimp is available in the literature. However, Shakila et al. (1995) demonstrated a general correlation between decreased sensory quality and increased

putrescine concentrations in shrimp. Cadaverine concentration did not seem to be correlated with sensory quality changes in their study however, these researchers reported the amine quantities in whole number mg% (one order of magnitude less sensitive detection level than parts per million) which resulted in the inability for these researchers to detect smaller amine changes which may have corresponded to sensory quality changes. Our research showed that increases in putrescine and cadaverine concentrations detected in parts per million were generally correlated to sensory quality changes.

Indole was not detected in aquacultured Nicaraguan shrimp decomposed at 0°C and it was not detected until two sample intervals (day 3.5) after sensory decomposition was determined at 12°C. Additionally, the maximum mean indole concentration for shrimp decomposed at 12°C was 4.1 µg/100g (day 4.5). This demonstrates severe limitations for indole concentration to be an effective chemical indicator of decomposition in shrimp decomposed at or below 12°C. Similar results were demonstrated by Chang et al. (1983) with shrimp decomposed on ice and at 4, 12, and 24°C however, in their study the indole level in the shrimp stored at 12°C increased to approximately 100 µg/100g by day 3. This discrepancy can again be explained by the fact that the shrimp used in our study were taken from the pond and placed directly in ice slush while still alive. Thus, indole-producing microorganisms, which generally grow at higher temperatures were severely limited in their opportunity for growth. Additionally, the shrimp used by Chang et al. (1983) were wild-caught Gulf of Mexico shrimp that were collected dockside. Therefore, the on-board handling procedures and complete temperature histories were unknown for the product. Improper on-board handling procedures may have contributed to contamination of the product with indole-producing

microorganisms and possible on-board temperature abuse may have allowed for subsequent indole production.

Mean indole concentrations ($\mu\text{g}/100\text{g}$) showed similar trends to putrescine and cadaverine concentrations (ppm) for aquacultured Nicaraguan shrimp decomposed at 24 and 36°C. The indole concentrations increased at a slightly slower rate than the putrescine and cadaverine concentrations but did increase above the 3- $\mu\text{g}/100\text{g}$ level at or near the point of sensory unacceptability at both temperatures. Indole was detected at the 3.3- $\mu\text{g}/100\text{g}$ level at hour 12 in shrimp decomposed at 24°C. This was one sample interval after sensory decomposition was declared on hour 10. At the 36°C storage temperature indole was detected in shrimp at the 3.3- $\mu\text{g}/100\text{g}$ level at hour 6 which corresponded to the time of sensory unacceptability. This demonstrates that an indole concentration above the 3- $\mu\text{g}/100\text{g}$ level was relatively effective as a chemical indicator of decomposition in aquacultured Nicaraguan shrimp decomposed at or above 24°C. Matches (1983), Chang et al. (1983), and Shamshad et al. (1990) have all demonstrated the effectiveness of indole concentration as a chemical indicator in shrimp decomposed at high temperatures of storage.

Based on the above discussion, it appears that putrescine concentration at the 3-ppm level was a distinct chemical indicator of decomposition in aquacultured Nicaraguan shrimp decomposed at temperatures ranging from 0-36°C. It showed only minor limitations at the 0°C storage temperatures and was generally very versatile over the temperature parameters tested. Cadaverine concentration at the 3-ppm level performed as well as putrescine concentration at the 3-ppm level when the shrimp were decomposed at or above 12°C. However, it was rather ineffective for shrimp decomposed at 0°C. Indole

concentration at the 3- $\mu\text{g}/100\text{g}$ level was generally an effective chemical indicator of decomposition for shrimp decomposed at or above 24°C but was clearly ineffective when the shrimp were decomposed at or below 12°C. Thus under the parameters tested in this research, putrescine concentration at the 3-ppm level was the best overall chemical indicator of decomposition in aquacultured Nicaraguan shrimp across all storage temperatures.

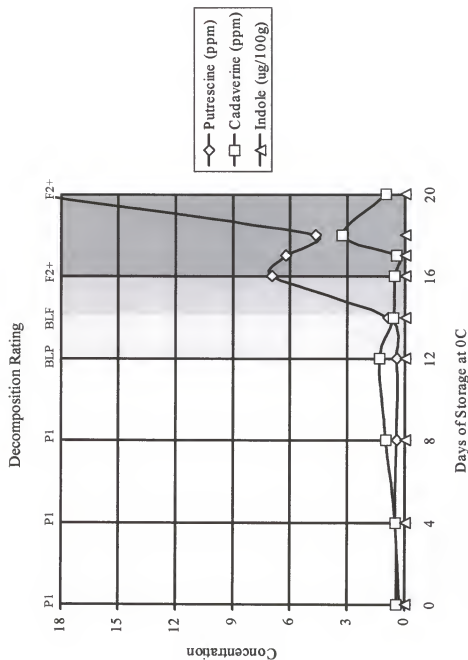


Figure 5 – Mean putrescine, cadaverine, and indole concentrations in aquacultured Nicaraguan Penaeid shrimp (*L. vannamei*) decomposed at 0°C with the y-axis reduced to focus on chemical changes occurring during the transition from acceptable to unacceptable product as judged by sensory evaluation. (Increased shading denotes progressive sensory decomposition; P1 = Pass Class 1, BLP = Borderline Pass, BLF = Borderline Fail, F2+ = Fail Class 2 and greater.)

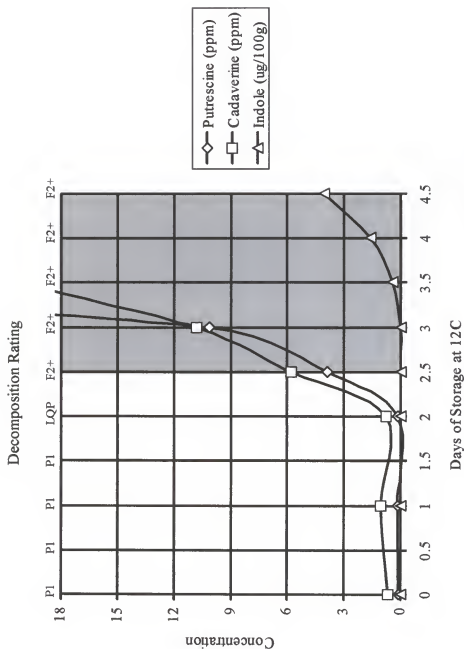


Figure 6 – Mean putrescine, cadaverine, and indole concentrations in aquacultured Nicaraguan Penaeid shrimp (*L. vannamei*) decomposed at 12°C with the y-axis reduced to focus on chemical changes occurring during the transition from acceptable to unacceptable product as judged by sensory evaluation. (Increased shading denotes progressive sensory decomposition; P1 = Pass Class 1, LQP = Low Quality Pass, F2+ = Fail Class 2 and greater.)

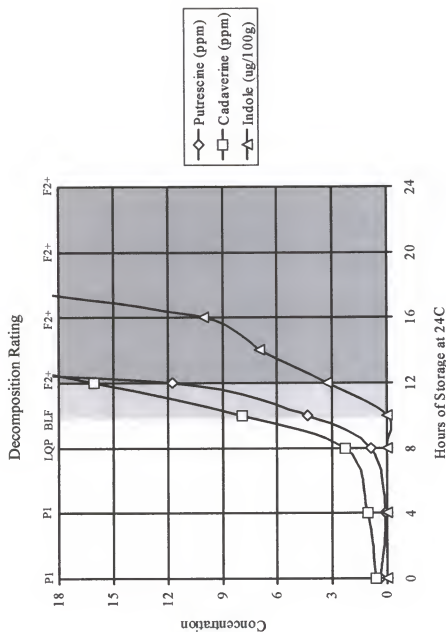


Figure 7 – Mean putrescine, cadaverine, and indole concentrations in aquacultured Nicaraguan Penaeid shrimp (*L. vannamei*) decomposed at 24°C with the y-axis reduced to focus on chemical changes occurring during the transition from acceptable to unacceptable product as judged by sensory evaluation. (Increased shading denotes progressive sensory decomposition; P1 = Pass Class 1, LQP = Low Quality Pass, BLF = Borderline Fail, F2+ = Fail Class 2 and greater.)

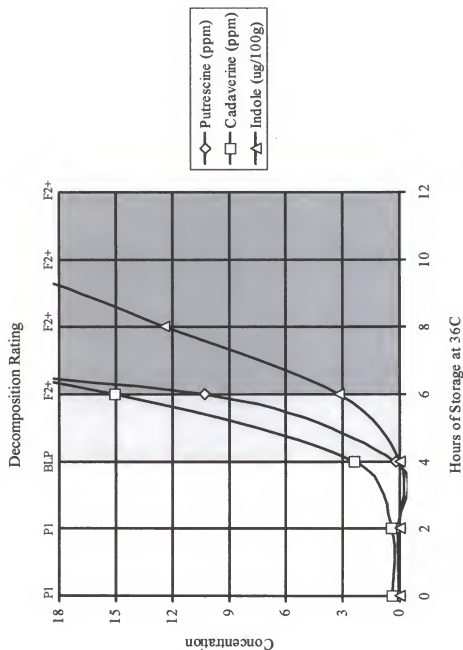


Figure 8 – Mean putrescine, cadaverine, and indole concentrations in aquacultured Nicaraguan Penaeid shrimp (*L. vannamei*) decomposed at 36°C with the y-axis reduced to focus on chemical changes occurring during the transition from acceptable to unacceptable product as judged by sensory evaluation. (Increased shading denotes progressive decomposition; P1 = Pass Class 1, BLP = Borderline Pass, F2+ = Fail Class 2 and greater.)

Statistical Analyses

Figures 9-12 are graphs of putrescine, cadaverine, and indole concentrations in aquacultured Nicaraguan Penaeid shrimp (*L. vannamei*) versus time of storage with nonlinear regression curves based on Mitcherlich's equation fit to each chemical indicator of decomposition at 0, 12, 24, and 36°C, respectively. No curve was fit to the indole concentrations at 0°C since no indole was detected in these samples. Nonlinear regression analyses based on Mitcherlich's equation were used because they displayed the best fit to these data. Mitcherlich's equation is an exponential equation that is useful in agriculture and chemistry when the variable of interest (i.e. putrescine, cadaverine, or indole) increases exponentially with time. Using Mitcherlich's equation, the concentration of a selected chemical indicator can be predicted relative to temperature and time (Myers, 1986).

The nonlinear regression curves fit to putrescine and cadaverine concentrations in the shrimp decomposed at 0°C (Figure 9) displayed R^2 values of 0.818 and -0.071, respectively. The nonlinear regression curves for shrimp decomposed at 0°C had the lowest R^2 values of all regression analyses run. Nonlinear regression analysis for putrescine concentrations predicted that the putrescine level would reach 3 ppm approximately two days after the shrimp were considered unacceptable (BLF) and just as the shrimp were rated F2. Thus, putrescine concentration at the 3-ppm level was predicted to be an effective chemical indicator of shrimp when the shrimp was decomposed at 0°C. Nonlinear regression analysis of cadaverine concentrations in these same shrimp resulted in a prediction equation with very poor fit that predicted only minimal increases in cadaverine levels during storage and a maximum level of

approximately 0.64 ppm. Thus, cadaverine concentration (especially at the 3-ppm level) was predicted to be an unreliable chemical indicator of decomposition for shrimp decomposed at 0°C. Indole concentration was, of course, useless as a chemical indicator since no indole was detected in shrimp decomposed at 0°C.

The nonlinear regression curves fit to putrescine, cadaverine, and indole concentrations in shrimp decomposed at 12°C (Figure 10) displayed R^2 values of 0.918, 0.889, and 0.098, respectively. Nonlinear regression analysis for putrescine concentrations predicted that the putrescine level would reach 3 ppm just before the shrimp were rated unacceptable (F2) by sensory analyses (day 2.5). Nonlinear regression analysis for cadaverine concentrations predicted that the cadaverine level would reach 3 ppm one sample increment (day 2) before the shrimp were rated unacceptable (F2) by sensory analyses. Both of these indicators continued to increase at a rapid rate as decomposition of the shrimp progressed. Consequently, the nonlinear regression analyses predicted that putrescine and cadaverine concentrations at the 3-ppm level would be effective indicators of decomposition for shrimp stored at 12°C. Indole concentration was an ineffective chemical indicator for shrimp decomposed at 12°C since no indole was detected in these samples until 24 hours after the shrimp were considered unacceptable (F2) by sensory criteria.

The nonlinear regression curves fit to putrescine, cadaverine, and indole concentrations in shrimp decomposed at 24°C (Figure 11) displayed R^2 values of 0.952, 0.941, and 0.809, respectively. Adjustments were made in the analyses of these data by disregarding the final two sample increments to allow for more precise curve fitting in the transition area of acceptable to unacceptable product. Nonlinear regression analysis for

putrescine concentrations predicted that the putrescine level would reach 3 ppm slightly before the sample was rated unacceptable (BLF) by sensory analysis (hour 10). Nonlinear regression analysis for cadaverine concentrations predicted that the cadaverine level would reach 3 ppm one sample increment (hour 8) before the sample was rated unacceptable (BLF) by sensory analysis. The nonlinear regression analysis for indole concentrations predicted that indole would reach the 3-ppm level two sample increments (hour 14) after the sample was rated unacceptable (BLF) by sensory analysis. Also, all three indicators continued to increase at a rapid rate as decomposition of the shrimp progressed. Thus, putrescine and cadaverine concentrations at the 3-ppm level were predicted to be effective chemical indicators while indole at the 3-ppm level was predicted to be a less accurate indicator of decomposition for shrimp decomposed at 24°C.

The nonlinear regression curves fit to putrescine, cadaverine, and indole concentrations in shrimp decomposed at 36°C (Figure 12) displayed R^2 values of 0.934, 0.946, and 0.933, respectively. Nonlinear regression analysis for putrescine concentrations predicted that the putrescine level would reach 3 ppm slightly before the shrimp were rated unacceptable (F2) by sensory analysis (hour 6). Nonlinear regression analysis for cadaverine concentrations predicted that the cadaverine level would reach 3 ppm approximately 2 hours before the samples were rated unacceptable (BLF) by sensory analysis. The nonlinear regression analysis for indole concentrations predicted that indole would reach the 3-ppm level shortly after the shrimp were rated unacceptable (BLF) by sensory analysis. Again, all three indicators continued to increase at a rapid rate as decomposition of the shrimp progressed. Consequently, putrescine, cadaverine,

and indole concentrations at the 3-ppm level were predicted to be effective chemical indicators of decomposition for shrimp decomposed at 36°C.

Based on the above analyses, putrescine concentration at the 3-ppm level, cadaverine concentration at the 3-ppm level, and indole concentration at the 3- μ g/100g level were chosen as the threshold concentrations at which each chemical indicator would be tested for its ability to predict/confirm FDA sensory decomposition ratings in contingency table analyses. Indole concentration at the traditional 25- μ g/100g level was also chosen for these analyses for comparative value.

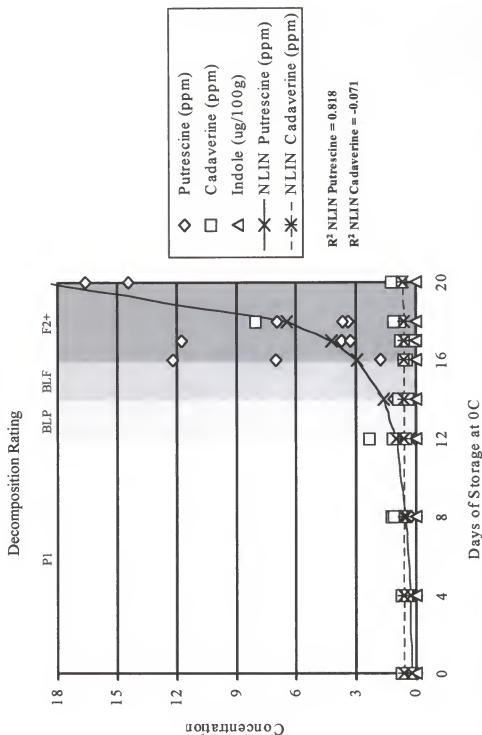


Figure 9 – Nonlinear regression analyses (Mitcherlich's equation) of putrescine, cadaverine, and indole concentrations in aquacultured Nicaraguan Penaeid shrimp (*L. vannamei*) decomposed at 0°C with the y-axis reduced to focus on chemical changes occurring during the transition from acceptable to unacceptable product as judged by sensory evaluation. (Increased shading denotes progressive sensory decomposition; P1 = Pass Class 1, BLF = Borderline Pass, BLF = Borderline Fail, F2+ = Fail Class 2 and greater.)

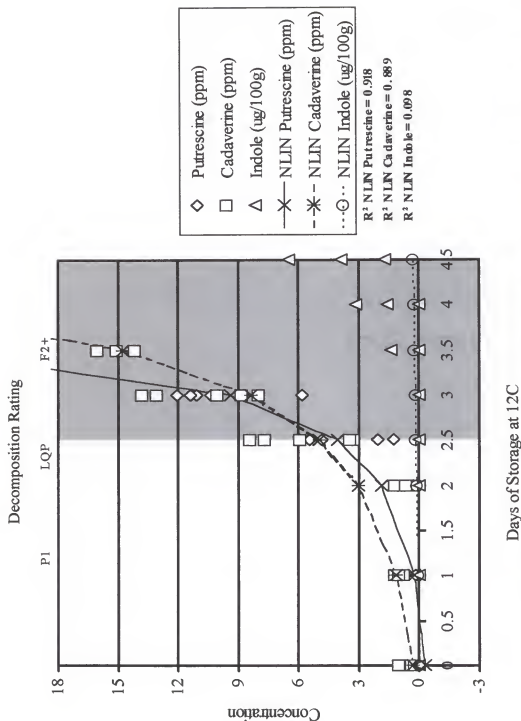


Figure 10 – Nonlinear regression analyses (Mitcherlich's equation) of putrescine, cadaverine, and indole concentrations in aquacultured Nicaraguan Penaeid shrimp (*L. vannamei*) decomposed at 12°C with the y-axis reduced to focus on chemical changes occurring during the transition from acceptable to unacceptable product as judged by sensory evaluation. (Increased shading denotes progressive sensory decomposition; P1 = Pass Class 1, LQP = Low Quality Pass, F2+ = Fail Class 2 and greater.)

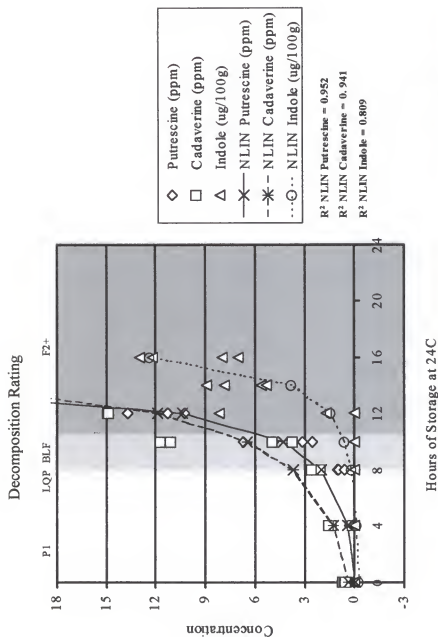


Figure 11 – Nonlinear regression analyses (Mitcherlich's equation) of putrescine, cadaverine, and indole concentrations in aquacultured Nicaraguan Penaeid shrimp (*L. vannamei*) decomposed at 24°C with the y-axis reduced to focus on chemical changes occurring during the transition from acceptable to unacceptable product as judged by sensory evaluation. (Increased shading denotes progressive sensory decomposition; P1 = Pass Class 1, LQP = Low Quality Pass, BLF = Borderline Fail, F2+ = Fail Class 2 and greater.)

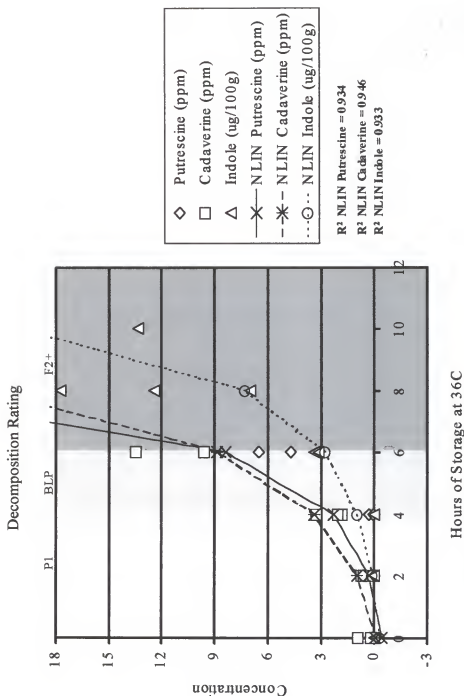


Figure 12 – Nonlinear regression analyses (Mitcherlich's equation) of putrescine, cadaverine, and indole concentrations in aquacultured Nicaraguan Penaeid shrimp (*L. vannamei*) decomposed at 36°C with the y-axis reduced to focus on chemical changes occurring during the transition from acceptable to unacceptable product as judged by sensory evaluation. (Increased shading denotes progressive sensory decomposition; P1 = Pass Class 1, BLP = Borderline Pass, F2+ = Fail Class 2 and greater.)

Tables 15-18 are contingency tables comparing the decomposition sensory rating (Pass vs. Fail) to putrescine concentration at the 3-ppm level (<3 ppm vs. >3 ppm), cadaverine concentration at the 3-ppm level (<3 ppm vs. >3 ppm), indole concentration at the 3- $\mu\text{g}/100\text{g}$ level (<3 $\mu\text{g}/100\text{g}$ vs. >3 $\mu\text{g}/100\text{g}$), and indole concentration at the 25- $\mu\text{g}/100\text{g}$ level (<25 $\mu\text{g}/100\text{g}$ vs. >25 $\mu\text{g}/100\text{g}$) for aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 0, 12, 24, and 36°C. Contingency tables were constructed to test the efficacy of selected levels of putrescine, cadaverine, and indole as chemical indicators of decomposition in shrimp. In these tables, the numeral in each cell indicates the number of samples included in each combination of indicator concentration and sensory rating. The unshaded cells represent situations in which the chemical concentration and the sensory rating agree relative to accepting or rejecting the product. Conversely, the shaded cells represent situations in which the chemical concentration and the sensory rating do not agree relative to accepting or rejecting the product.

Measures for sensitivity, specificity, and predictive value were calculated based on contingency table values and correlation between the FDA sensory classifications and selected concentrations of putrescine, cadaverine, and indole was tested using the Kendall Partial Rank Correlation Coefficient (Kendall's Tau) (Siegel, 1956). Sensitivity measures the probability that the chemical indicator at the selected level will predict (confirm) decomposition when the sample fails sensory analysis while specificity measures the probability that the chemical indicator at the selected level will not predict decomposition when the sample passes sensory analysis. Predictive value measures the probability that the sample will fail sensory analysis when the chemical indicator predicts

decomposition. And finally, Kendall's Tau (The Kendall Partial Rank Correlation Coefficient) is a measure of the correlation between FDA sensory classifications and the selected concentrations of putrescine, cadaverine, and indole. It should be noted that the two most useful statistics that can be applied to the data in this study are sensitivity and predictive since in practice (regulatory and industry) shrimp samples generally undergo sensory analysis prior to chemical analysis. In the regulatory case, samples that pass sensory analysis are rarely chemically tested while samples that fail sensory analysis are commonly tested chemically to determine the legal action taken and to produce evidence for such legal action.

Putrescine concentration at the 3-ppm level agreed with the decomposition sensory rating for aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 0, 12, 24, and 36°C in 94 of the 98 samples evaluated resulting in an error rate of 4.1% (Table 15). Sensitivity, specificity, predictive value, and Kendall's Tau calculations from this contingency table produced values of 0.95, 0.98, 0.98, and 0.92, respectively.

Cadaverine concentration at the 3-ppm level agreed with the decomposition sensory rating for aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 0, 12, 24, and 36°C in 81 of the 98 samples evaluated resulting in an error rate of 17.4% (Table 16). Sensitivity, specificity, predictive value, and Kendall's Tau calculations from this contingency table produced values of 0.77, 0.90, 0.91, and 0.67, respectively.

Indole concentration at the 3-μg/100g level agreed with the decomposition sensory rating for aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 0, 12, 24, and 36°C in 70 of the 98 samples evaluated resulting in an error rate of 28.6% (Table

17). Sensitivity, specificity, predictive value, and Kendall's Tau calculations from this contingency table produced values of 0.50, 1.0, 1.0, and 0.55, respectively.

Indole concentration at the 25- μ g/100g level agreed with the decomposition sensory rating for aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 0, 12, 24, and 36°C in only 51 of the 98 samples evaluated resulting in an error rate of 48.0% (Table 18). Sensitivity, specificity, predictive value, and Kendall's Tau calculations from this contingency table produced values of 0.16, 1.0, 1.0, and 0.28, respectively.

These results clearly showed that putrescine concentration at the 3-ppm level accurately confirmed sensory decomposition in shrimp decomposed at temperatures ranging from 0-36°C. Cadaverine concentration at the 3-ppm level, indole concentration at the 3- μ g/100g level, and indole concentration at the 25- μ g/100g level confirmed sensory decomposition with diminishing accuracy relative to putrescine at the 3-ppm level.

Comparisons of indicator concentrations to sensory ratings for shrimp decomposed at 0°C (data not shown) clearly show that putrescine concentration at the 3-ppm level performed markedly better than cadaverine concentration at the 3-ppm level, indole concentration at the 3- μ g/100g level, and indole concentration at the 25- μ g/100g level when the shrimp were decomposed at 0°C. Putrescine concentration at the 3-ppm level disagreed with the sensory rating of the shrimp in only 3 of 26 samples evaluated while 13, 14, and 14 cases of disagreement occurred for cadaverine concentration at the 3-ppm level, indole concentration at the 3- μ g/100g level, and indole concentration at the 25- μ g/100g level, respectively.

Results comparing indicator concentrations to sensory ratings for shrimp decomposed at 12°C (data not shown) clearly identify putrescine concentration at 3 ppm and cadaverine concentration at 3 ppm as excellent chemical indicators of decomposition for the shrimp decomposed at this temperature. Putrescine concentration at the 3-ppm level agreed with the sensory rating in all samples while cadaverine concentration at the 3-ppm level agreed with the sensory in 23 out of 24 samples evaluated. Indole concentrations at the 3- $\mu\text{g}/100\text{g}$ and 25- $\mu\text{g}/100\text{g}$ level were not effective as chemical indicators of decomposition in shrimp decomposed at 12°C showing disagreements with sensory ratings on 11 and 14 samples, respectively.

Comparisons of indicator concentrations to sensory ratings for shrimp decomposed at 24°C (data not shown) again identify putrescine concentration at 3 ppm and cadaverine concentration at 3 ppm as excellent chemical indicators of decomposition for the shrimp decomposed at 24°C. Putrescine concentration at the 3-ppm level agreed with the sensory rating in 26 of 27 samples while cadaverine concentration at the 3-ppm level agreed with the sensory ratings in 25 out of 27 samples evaluated. Indole concentration at the 3- $\mu\text{g}/100\text{g}$ level performed much better as a chemical indicator of shrimp decomposed at 24°C than it did for the shrimp decomposed at 0 and 12°C showing agreement with sensory ratings in 23 of 26 samples tested. However, indole concentration at the 25- $\mu\text{g}/100\text{g}$ level was an ineffective chemical indicator of decomposition in shrimp decomposed at 24°C showing disagreement with sensory ratings in 11 of 27 samples.

Results comparing indicator concentrations to sensory ratings for shrimp decomposed at 36°C (data not shown) clearly identify putrescine concentration at 3 ppm,

cadaverine concentration at 3 ppm, and indole concentration at 3 µg/100g as excellent chemical indicators of decomposition for the shrimp decomposed at this temperature. Putrescine concentration at the 3-ppm level agreed with the sensory ratings in all 21 samples, cadaverine concentration at the 3-ppm level agreed with the sensory ratings in 20 out of 21 samples, and indole concentration at the 3-µg/100g level agreed with the sensory ratings in all 21 samples evaluated. Indole concentration at the 25-µg/100g level was ineffective as a chemical indicator of decomposition in shrimp decomposed at 36°C showing disagreement with sensory ratings in 8 of the 21 samples evaluated.

Table 15 - Contingency table comparing the decomposition sensory rating to putrescine concentration (ppm) for aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 0, 12, 24, and 36°C^{a,b,c}

Concentration	Sensory Rating	
	Pass	Fail
< 3 ppm	41	3
> 3 ppm	1	53

^aThe numeral in each cell indicates the number of samples included in each concentration/sensory rating combination.

^bThe shaded cells represent disagreements between sensory ratings and the 3 ppm putrescine concentration in the shrimp.

^cSensitivity = 0.95, Specificity = 0.98, Predictive value = 0.98, Kendall's Tau = 0.92

Table 16 - Contingency table comparing the decomposition sensory rating to cadaverine concentration (ppm) for aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 0, 12, 24, and 36°C^{a,b,c}

Concentration	Sensory Rating	
	Pass	Fail
< 3 ppm	38	13
> 3 ppm	4	43

^aThe numeral in each cell indicates the number of samples included in each concentration/sensory rating combination.

^bThe shaded cells represent disagreements between sensory ratings and the 3 ppm cadaverine concentration in the shrimp.

^cSensitivity = 0.77, Specificity = 0.90, Predictive value = 0.91, Kendall's Tau = 0.67

Table 17 - Contingency table comparing the decomposition sensory rating to indole concentration ($\mu\text{g}/100\text{g}$) for aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 0, 12, 24, 36°C^{a,b,c}

Concentration	Sensory Rating	
	Pass	Fail
< 3 $\mu\text{g}/100\text{g}$	42	28
> 3 $\mu\text{g}/100\text{g}$	0	28

^aThe numeral in each cell indicates the number of samples included in each concentration/sensory rating combination.

^bThe shaded cells represent disagreements between sensory ratings and the 3 $\mu\text{g}/100\text{g}$ indole concentration in the shrimp.

^cSensitivity = 0.50, Specificity = 1, Predictive value = 1, Kendall's Tau = 0.55

Table 18 - Contingency table comparing the decomposition sensory rating to indole concentration ($\mu\text{g}/100\text{g}$) for aquacultured Nicaraguan shrimp (*L. vannamei*) decomposed at 0, 12, 24, 36°C^{a,b,c}

Concentration	Sensory Rating	
	Pass	Fail
< 25 $\mu\text{g}/100\text{g}$	42	47
> 25 $\mu\text{g}/100\text{g}$	0	9

^aThe numeral in each cell indicates the number of samples included in each concentration/sensory rating combination.

^bThe shaded cells represent disagreements between sensory ratings and the 25 $\mu\text{g}/100\text{g}$ indole concentration in the shrimp.

^cSensitivity = 0.16, Specificity = 1, Predictive value = 1, Kendall's Tau = 0.28

FDA Commercial Shrimp

Chemical and Sensory Analyses

Putrescine, cadaverine, and indole levels in commercial shrimp samples collected and evaluated by the FDA Pacific Regional Laboratory (Bothell, WA) are shown in Table 19. Forty-four samples imported from seven countries including China, Chile, India, Indonesia, Pakistan, Thailand, and Macau were tested. Twenty-nine of the 44 samples were of unacceptable sensory quality as determined by FDA sensory experts. Of these 29 unacceptable samples only 3 samples had putrescine concentrations lower than 3 ppm,

which demonstrates excellent effectiveness for putrescine at the 3-ppm level to confirm sensory decomposition in commercial shrimp samples. Twenty-one of the 29 unacceptable samples displayed cadaverine concentrations lower than 3 ppm demonstrating a marked ineffectiveness for cadaverine concentration at the 3-ppm level to confirm sensory decomposition. However, 23 of the 29 unacceptable samples had cadaverine concentrations greater than 1.5 ppm, which suggests the possibility that a lower cadaverine concentration could be used to confirm sensory decomposition in commercial shrimp. Twenty-three of the 29 unacceptable samples had indole concentrations greater than 3 $\mu\text{g}/100\text{g}$ while only 9 had indole concentrations greater than 25 $\mu\text{g}/100\text{g}$. This data showed that indole concentration at the 3- $\mu\text{g}/100\text{g}$ level was clearly a more effective chemical indicator of decomposition than indole concentration at the 25- $\mu\text{g}/100\text{g}$ level for commercial shrimp samples. Overall, the data showed that putrescine at the 3-ppm level was the most accurate chemical indicator to confirm decomposition in the commercial shrimp samples tested.

Table 19 – Putrescine, cadaverine, and indole levels in commercial shrimp samples collected and evaluated for decomposition by the FDA Pacific Regional Laboratory (Bothell, WA)

Sample Identification	FDA Sensory Classification	Putrescine (ppm)	Cadaverine (ppm)	Indole (µg/100g)
China P&D ^a tail-on	Pass	1.24	1.58	1.76
China P&D tail-on	Pass	14.48	3.84	1.26
China P&D tail-on	Pass	0.68	0	1.37
China PUD ^b	Pass	NA	NA	3.93
China PUD	Pass	NA ^d	NA	0
China PUD tail-on	Pass	0.46	0.14	0
Chile PUD	Pass	0	2.62	1.82
India P&D	Pass	NA	NA	2.47
India Peeled	Pass	2.02	1.72	8.55
Indonesia Peeled	Pass	3.5	0	4.40
Indonesia Peeled	Pass	3.56	0	3.81
Indonesia Peeled	Pass	NA	NA	5.08
Pakistan Peeled	Pass	NA	NA	19.60
Pakistan PUD	Pass	1.84	1.62	7.92
Thailand Peeled tail-on	Pass	10.14	2.7	7.62
China P&D tail-on	Fail	8.2	0.54	1.72
China PUD	Fail	6.04	3.34	1.88
China PUD	Fail	171.64	10.28	27.94
China PUD tail-on	Fail	0.4	0.12	0
China PUD tail-on	Fail	3.4	2.04	0.75
Chile PUD	Fail	5.68	2.32	116.01
India P&D	Fail	4.32	2.44	12.52
India Peeled	Fail	6.48	3.94	24.75
Indonesia Peeled	Fail	21.2	1.34	21.86
Indonesia Peeled	Fail	82.9	5.9	48.11
Pakistan Peeled	Fail	19.22	2.12	31.48
Pakistan Peeled	Fail	9.2	0.46	45.03
Pakistan Peeled	Fail	14.82	3.44	66.23
Pakistan PUD	Fail	3.1	1.9	17.10
Pakistan PUD	Fail	4.3	2.08	41.11
Thailand Peeled tail-on	Fail	12.7	4.7	14.98
China PUD	Fail	2.24	1.6	2.55
China PUD tail-on	Fail	7.34	2.86	4.71
China PUD	Fail	11.18	11.88	6.95
India P&D	Fail	7.42	1.62	24.11
India P&D	Fail	10.8	2.24	26.08
India PUD	Fail	34.24	3.84	19.53
India P&D	Fail	6.14	1.74	10.52

Table 19 – continued

Sample Identification	FDA Sensory Classification	Putrescine (ppm)	Cadaverine (ppm)	Indole (µg/100g)
India PUD tail-on	Fail	5.9	1.52	34.84
India C&P ^c	Fail	3.24	0.54	19.08
Indonesia P&D	Fail	22.52	1.66	21.87
Indonesia PUD	Fail	18.82	1.54	5.42
Indonesia PUD	Fail	48.22	1.66	18.63
Macau PUD	Fail	1.32	0	2.05

^aP&D = Peeled and Deveined^bPUD = Peeled and Undeveined^cC&P = Cooked and Peeled^dNA = Data not available

Statistical Analyses

Tables 20-23 are contingency tables comparing the decomposition sensory rating (Pass vs. Fail) to putrescine concentration at the 3-ppm level (<3 ppm vs. >3 ppm), cadaverine concentration at the 3-ppm level (<3 ppm vs. >3 ppm), indole concentration at the 3-µg/100g level (<3 µg/100g vs. >3 µg/100g), and indole concentration at the 25-µg/100g level (<25 µg/100g vs. >25 µg/100g) level for commercial shrimp samples collected and evaluated by the FDA Pacific Regional Laboratory (Bothell, WA).

Putrescine concentration at the 3-ppm level agreed with the decomposition sensory rating in 32 of 39 samples tested resulting in an error rate of 18.0% (Table 20). Sensitivity, specificity, predictive value, and Kendall's Tau calculations from this contingency table produced values of 0.90, 0.60, 0.87, and 0.51, respectively.

Cadaverine concentration at the 3-ppm level agreed with the decomposition sensory rating in only 17 of the 39 commercial samples resulting in an error rate of 56.4% (Table 21). Sensitivity, specificity, predictive value, and Kendall's Tau calculations from this contingency table produced values of 0.28, 0.90, 0.89, and 0.18, respectively.

Indole concentration at the 3- $\mu\text{g}/100\text{g}$ level agreed with the decomposition sensory rating for the commercial shrimp samples in 30 of the 44 samples evaluated resulting in an error rate of 31.8% (Table 22). Sensitivity, specificity, predictive value, and Kendall's Tau calculations from this contingency table produced values of 0.79, 0.47, 0.74, and 0.27, respectively.

Indole concentration at the 25- $\mu\text{g}/100\text{g}$ level agreed with the decomposition sensory rating for the commercial samples in only 24 of the 44 samples tested resulting in an error rate of 45.5% (Table 23). Sensitivity, specificity, predictive value, and Kendall's Tau calculations from this contingency table produced values of 0.31, 1.0, 1.0, 0.36, respectively.

The data clearly showed that putrescine concentration at the 3-ppm level most accurately confirmed sensory decomposition in the FDA commercial samples. While indole concentration at the 3- $\mu\text{g}/100\text{g}$ level, indole concentration at the 25- $\mu\text{g}/100\text{g}$ level, and cadaverine concentration at the 3-ppm level confirmed sensory decomposition with diminishing accuracy relative to putrescine at the 3-ppm level.

Although the error rate of 18.0% for putrescine concentration at the 3-ppm level seems rather high, only 3 of the 29 commercial samples tested showed putrescine concentrations of less than 3 ppm when the sample failed sensory analysis (Sensitivity = 0.90). This means, as stated previously, that of the 29 samples that failed sensory analysis only 3 samples would not be chemically confirmed by putrescine concentration at the 3-ppm level. The 4 samples that passed sensory evaluation but had putrescine concentrations greater than 3 ppm (Specificity = 0.60) likely reflect decomposed product

that did not display definite odors of decomposition and thus were considered acceptable by the sensory analysts.

Table 20 - Contingency table comparing the decomposition sensory rating to putrescine concentration (ppm) for commercial shrimp samples collected and evaluated for decomposition by the FDA Pacific Regional Laboratory (Bothell, WA)^{a,b,c}

Concentration	Sensory Rating	
	Pass	Fail
< 3 ppm	6	3
> 3 ppm	4	26

^aThe numeral in each cell indicates the number of samples included in each concentration/sensory rating combination.

^bThe shaded cells represent disagreements between sensory ratings and the 3 ppm putrescine concentration in the shrimp.

^cSensitivity = 0.90, Specificity = 0.60, Predictive value = 0.87, Kendall's Tau = 0.51

Table 21 - Contingency table comparing the decomposition sensory rating to cadaverine concentration (ppm) for commercial shrimp samples collected and evaluated for decomposition by the FDA Pacific Regional Laboratory (Bothell, WA)^{a,b,c}

Concentration	Sensory Rating	
	Pass	Fail
< 3 ppm	9	21
> 3 ppm	1	8

^aThe numeral in each cell indicates the number of samples included in each concentration/sensory rating combination.

^bThe shaded cells represent disagreements between sensory ratings and the 3 ppm cadaverine concentration in the shrimp.

^cSensitivity = 0.28, Specificity = 0.90, Predictive value = 0.89, Kendall's Tau = 0.18

Table 22 - Contingency table comparing the decomposition sensory rating to indole concentration ($\mu\text{g}/100\text{g}$) for commercial shrimp samples collected and evaluated for decomposition by the FDA Pacific Regional Laboratory (Bothell, WA)^{a,b,c}

Concentration	Sensory Rating	
	Pass	Fail
< 3 $\mu\text{g}/100\text{g}$	7	6
> 3 $\mu\text{g}/100\text{g}$	8	23

^aThe numeral in each cell indicates the number of samples included in each concentration/sensory rating combination.

^bThe shaded cells represent disagreements between sensory ratings and the 3 $\mu\text{g}/100\text{g}$ indole concentration in the shrimp.

^cSensitivity = 0.79, Specificity = 0.47, Predictive value = 0.74, Kendall's Tau = 0.27

Table 23 - Contingency table comparing the decomposition sensory rating to indole concentration ($\mu\text{g}/100\text{g}$) for commercial shrimp samples collected and evaluated for decomposition by the FDA Pacific Regional Laboratory (Bothell, WA)^{a,b,c}

Concentration	Sensory Rating	
	Pass	Fail
< 25 $\mu\text{g}/100\text{g}$	15	20
> 25 $\mu\text{g}/100\text{g}$	0	9

^aThe numeral in each cell indicates the number of samples included in each concentration/sensory rating combination.

^bThe shaded cells represent disagreements between sensory ratings and the 25 $\mu\text{g}/100\text{g}$ indole concentration in the shrimp.

^cSensitivity = 0.31, Specificity = 1, Predictive value = 1, Kendall's Tau = 0.36

CONCLUSIONS

Within the limits of the experimental procedures used and the number of samples analyzed, the following conclusions can be drawn based on staged experiments and field verification from FDA commercial samples:

1. Putrescine concentration at the 3-ppm level accurately confirmed sensory decomposition in shrimp decomposed at temperatures ranging from 0-36°C and in field tests on commercial shrimp samples collected and evaluated by FDA experts.
2. Cadaverine concentration at the 3-ppm level did not accurately confirmed sensory decomposition in shrimp decomposed at temperatures ranging from 0-36°C or in field tests on commercial shrimp samples collected and evaluated by FDA experts.
3. Indole concentration at the 3-µg/100g and 25-µg/100g levels did not accurately confirm decomposition in shrimp decomposed at temperatures ranging from 0-36°C or in field tests on commercial shrimp samples collected and evaluated by FDA experts.
4. The numbers and types of microorganisms responsible for the production of putrescine, cadaverine, and indole in shrimp decomposed at temperatures ranging from 0-36°C increased with decomposition temperature and time.

5. *Shewanella putrefaciens* was the single most prominent microorganism isolated from shrimp decomposed at temperatures ranging from 0-36°C that showed a positive association with putrescine concentrations.

Overall, this study demonstrated that putrescine concentration at the 3-ppm level was the most effective chemical indicator for decomposition in shrimp decomposed under controlled conditions at temperatures ranging from 0-36°C and in commercial shrimp samples collected and evaluated by FDA experts. It consistently confirmed sensory decomposition more accurately than cadaverine concentration at 3 ppm, indole concentration at 3 µg/100g, and indole concentration at 25 µg/100g in controlled decomposition experiments and in field verifications. Additionally, further research is necessary to determine why cadaverine concentration at 3 ppm was less effective in predicting sensory decomposition in FDA commercial shrimp samples than it was for shrimp from controlled decomposition experiments. Thus, it is recommended that putrescine concentration at the 3-ppm level be incorporated in industry and regulatory analyses to confirm (pass vs. fail) sensory decomposition in Penaeid shrimp.

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BIOGRAPHICAL SKETCH

Ronald Allen Benner, Jr. was born in Houston, TX, on November 21, 1964. After living in several Texas coastal communities he graduated in 1983 as Salutatorian from St. Joseph High School in Victoria, TX. From there he attended Victoria College and then transferred to Texas A&M University where he graduated with a B.B.A. in marketing in 1988. After working for two years he again enrolled at Texas A&M University where he received a Master of Science degree specializing in food microbiology in 1993. From 1993 through 1996 he gained experience in the seafood industry, the analytical food testing industry, and the food and beverage can manufacturing industry. Subsequently, in January 1997 he enrolled at the University of Florida and began pursuit of his Ph.D. specializing in seafood quality and safety under the guidance of Dr. W. Steven Otwell and Dr. Doug Archer. He plans to graduate in May 2001 and pursue employment interests.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



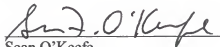
Walker S. Otwell, Chair
Professor of Food Science and
Human Nutrition

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Doug Archer
Professor of Food Science and
Human Nutrition

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



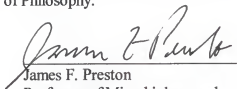
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Ramon C. Littell
Professor of Statistics

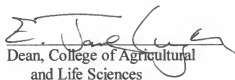
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This dissertation was submitted to the Graduate Faculty of the College of Agricultural and Life Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May, 2001



Dean, College of Agricultural
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